Published in final edited form as:
Glia. 2009 August 1; 57(10): 1062–1071. doi:10.1002/glia.20829.

Adult-Onset Deficiency in Growth Hormone and Insulin-Like Growth Factor-I Alters Oligodendrocyte Turnover in the Corpus Callosum

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Abstract

Growth hormone (GH) and insulin-like growth factor-I (IGF-I) provide trophic support during development and also appear to influence cell structure, function and replacement in the adult brain. Recent studies demonstrated effects of the GH/IGF-I axis on adult neurogenesis, but it is unclear whether the GH/IGF-I axis influences glial turnover in the normal adult brain. In the current study we used a selective model of adult-onset GH and IGF-I deficiency to evaluate the role of GH and IGF-I in regulating glial proliferation and survival in the adult corpus callosum. GH/IGF-I-deficient dwarf rats of the Lewis strain were made GH/IGF-I replete via twice daily injections of GH starting at postnatal day 28 (P28), approximately the age at which GH pulse amplitude increases in developing rodents. GH/IGF-I deficiency was initiated in adulthood by removing animals from GH treatment. Quantitative analyses revealed that adult-onset GH/IGF-I deficiency decreased cell proliferation in the white matter and decreased the survival of newborn oligodendrocytes. These findings are consistent with the hypothesis that aging-related changes in the GH/IGF-I axis produce deficits in ongoing turnover of oligodendrocytes, which may contribute to aging-related cognitive changes and deficits in remyelination after injury.

Keywords

white matter; trophic factor; oligodendrocytes; demyelination; aging

INTRODUCTION

Declines in circulating levels of GH and IGF-I are robust hallmarks of aging in mammals (e.g., Sonntag et al., 2005; Sherlock and Toogood, 2007). Recent studies indicate that the neurobiological consequences of the decline in GH/IGF-I include decreased neurogenesis in the dentate gyrus of the hippocampus (Lichtenwalner et al., 2001; 2006), where IGF-I appears to affect primarily the survival of newborn neurons, but also may influence the maturation and differentiation of newborn cells (Aberg et al., 2000; Darnaudéry et al.,

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2006). The effects of the GH/IGF-I axis on cell turnover in the adult brain probably are not limited to neuronal progenitors, since IGF-I can promote proliferation of oligodendrocyte progenitor cells (OPCs) and differentiation and survival of oligodendrocytes (e.g., McMorris and McKinnon, 1996; Mason et al., 2000; Aberg et al., 2007; Pang et al., 2007). It is reasonable to hypothesize that the aging-related decline in GH/IGF-I and dependent changes in oligodendrocyte genesis and/or maturation may contribute to impaired remyelination in the central nervous system of aged individuals (Gilson and Blakemore, 1993; Shields et al., 1999; Franklin et al., 2002; Sim et al., 2002) and to a decline in normal cognitive function.

Evaluating the impact of circulating growth factors like GH and IGF-I on the brain during aging is complicated by the many neurobiological changes that occur with aging. Among studies of the effects of IGF-I and regulatory members of the IGF system on oligodendrocytes and OPCs, most have examined effects *in vitro* (e.g., McMorris et al., 1986; Kühl et al., 2002; Mason and Goldman, 2002; Cui and Almazan, 2007), during development (e.g., Goddard et al., 1999; Ye et al., 2004; Zeger et al., 2007), following neural injury (e.g., Mason et al., 2000; Genoud et al., 2005; Kumar et al., 2007; Pang et al., 2007; Wood et al., 2007; Ye et al. 2007; Chesik et al., 2008), and/or in transgenic models in which IGF-dependent effects on brain development limit assessment of effects in adult animals (Ye and D'Ercole, 1999; Ye et al., 2000; 2007; Genoud et al., 2005). To evaluate specifically the role of the GH/IGF-I axis in the regulation of ongoing OPC proliferation and oligodendrocyte survival and differentiation in the white matter of adult rats, we used a model of selective, adult-onset GH/IGF-I deficiency in dwarf (*dw/dw*) rats derived from the Lewis strain (Charlton et al., 1988; Carter et al., 2002). A spontaneous mutation in these rats causes a selective, 90% reduction in pituitary production of GH and a reduction in plasma IGF-I (Charlton et al., 1988), comparable in magnitude to the decline seen in aging (e.g., Breese et al., 1991; Sonntag et al., 1992). The specificity of this model and its similarity to the aging-related changes in pituitary hormones stand in contrast to other dwarf rodents (Cheng et al., 1983) and to hypophysectomized animals (Schoenle et al., 1982; Aberg et al., 2000; 2007), in which multiple pituitary hormones are depleted. Thus, *dw/dw* rats provide an excellent model for testing whether normal GH and IGF-I levels in the plasma are required to sustain normal glial genesis in adults.

This study assessed the effects of GH and IGF-I deficiency on several aspects of glial turnover. To evaluate proliferation of progenitors, dividing cells were labeled with bromodeoxyuridine (BrdU) after two weeks of GH/IGF-I deficiency and then the rats were perfused 18 hours later. In other animals a cohort of newborn cells was labeled with BrdU immediately prior to initiation of GH/IGF-I deficiency. We then analyzed the survival and maturation of those cells after a four week period of GH/IGF-I deficiency, using antibodies to BrdU and markers of oligodendrocytes, in order to test whether GH/IGF-I deficiency reduced the survival of BrdU-labeled cells and/or altered their differentiation and maturation.

MATERIALS AND METHODS

Animals and treatment with growth hormone

The breeding and treatment of *dw/dw* rats has been described previously (Lichtenwalner et al., 2006). In brief, male *dw/dw* pups were bred from *dw/+* females and *dw/dw* males, identified by significantly reduced body weight at 28 days of age, and injected twice daily (subcutaneously, 8:00, 16:00) with porcine GH (200 μg; provided by Dr. A.F. Parlow, UCLA Harbor Medical Center) starting at P28 (approximately 3 days after pulsatile secretion of GH normally begins [see Edén, 1979; Gabriel et al., 1992; LeRoith et al., 2001] and the youngest age at which dwarf animals can be reliably identified). At P98 one half of the rats were switched to saline injections (GH/IGF-I deficient) while the remaining animals

continued to receive GH twice daily (GH/IGF-I replete). Plasma samples were taken at the time of perfusion and analyzed by radioimmunoassay to confirm the deficiency-induced reduction in circulating IGF-I levels. The body weight of each rat was recorded weekly. Two groups of male dw/+ heterozygous litter mates (n = 8 Proliferation group, n=8 Survival group) were monitored for parameters supporting model validation, body weight gain and plasma IGF-I levels (Lichtenwalner et al., 2006).

S-phase labeling

To label a cohort of newborn cells, rats received three subcutaneous injections of BrdU (50 mg/kg body weight, in sterile 0.9% NaCl plus 0.007 N NaOH) administered two hours apart. Animals in the Proliferation group received BrdU injections on P111, two weeks after the onset of GH/IGF-I deficiency on P98, and were perfused 18 hours after the last BrdU injection to assess whether two weeks of GH/IGF-I deficiency affected the baseline rate of proliferation in the corpus callosum (CC). Rats in the Survival group received BrdU injections on P98 and then were perfused four weeks later on P126. Importantly, all animals in the Survival group were GH/IGF-I replete at the time of labeling of newborn cells but the subsequent survival and differentiation of newly generated cells occurred under conditions of either GH/IGF-I deficiency or repletion.

Tissue preparation and Immunolabeling

Each animal was sacrificed by anesthetic overdose (sodium pentobarbital, 150 mg/kg body weight) and perfused with 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4). A complete series of coronal cryostat sections of the brain (40 μm) from bregma −1.50 to −7.5 (Paxinos and Watson, 1998) were collected in cryoprotectant (25% glycerol, 25% ethylene glycol in 0.1M phosphate buffer, pH 7.4) and stored at −20°C until processed for immunohistochemistry (IHC) or immunofluorescence (IF). The density of BrdU-labeled cells was analyzed in sections labeled by IHC using rat monoclonal anti-BrdU (2.5 μg/ml; Accurate Chemical & Scientific Corp., see Lichtenwalner et al., 2006).

For analysis of OPCs, sections from rats in the Proliferation group were labeled by IF using rat anti-BrdU and rabbit anti-platelet-derived growth factor receptor-α (PDGFR-α; Santa Cruz (C-20); 0.2 μg/ml). For analysis of oligodendrocyte turnover, sections from rats in the Survival group were labeled with rat anti-BrdU and two oligodendrocyte markers: rabbit anti-glutathione S-transferase pi (anti-GSTpi, Novocastra; 1:150; Gotts and Chesselet, 2005) and mouse anti-adenomatous polyposis coli (anti-APC, EMD Biosciences Calbiochem®; 1 μg/ml; Bhat et al., 1996). GSTpi and APC have been used widely as markers of mature oliogodendrocytes and also appear to recognize oligodendrocytes early in their differentiation (e.g., Bhat et al., 1996; Kumar et al., 2007; Tamura et al. 2007). Immunolabeling for BrdU and cell type specific antibodies was visualized using secondary antibodies conjugated to Alexa 488, Alexa 633 (Invitrogen Corp., Molecular Probes®) and Cy3 (Jackson ImmunoResearch Laboratories, Inc.).

Quantification

All analyses were performed blindly using coded sections. A modification of the optical disector method (Gundersen et al., 1988; Kempermann et al., 1998) was used for quantification of cell proliferation and survival. Since BrdU labeled cells were not distributed homogenously, often occurring in clusters, the material in this study was not well suited for stereological determination of the total number of BrdU labeled cells in each region of interest (ROI). We counted all of the BrdU-labeled cells in the CC in series of systematically randomly selected sections through a portion of the anterior-posterior extent of the hippocampus (bregma −1.6 to −5.3, Paxinos and Watson, 1998), analyzing three sections from each rat in the Proliferation group and five sections from each rat in the

Survival group. Using the Neurolucida system for quantitative morphometry (Microbrightfield, Inc., Colchester, VT), BrdU-labeled cells were counted within the CC in each section, excluding cells in the top focal plane to avoid overestimation. To estimate the total density of BrdU-labeled cells in the CC, the sum of cell counts for all sections was divided by the total volume of the CC analyzed in each animal and counts of $BrdU^+$ cells presented as number per unit volume. Mean values were calculated for each group.

In order to test for effects of GH/IGF-I deficiency on glial turnover in gray matter, BrdU-labeled cells were analyzed in pyriform cortex. Although the borders of most cortical regions are indistinct and difficult to identify consistently, it was possible to reproducibly define a region comprising primarily pyriform cortex using only three points: 1) the cortical surface radially above the ventral-medial margin of the layer of superficial pyramidal neurons that characterizes pyriform cortex (easily visualized using differential-interference contrast optics), 2) the center of the rhinal fissure, and 3) the superficial border of the subcortical white matter radially beneath the center of the rhinal fissure. Points 1 and 2 were connected along the pial surface and points 2 and 3 were connected by a straight line. For rats in both the Proliferation and Survival groups, BrdU-labeled cells were counted in three sections representing the same anterior-posterior extent defined for the analysis of the CC (N=5 rats per condition).

For analysis of the differentiation of BrdU-labeled cells in the CC, sections labeled for BrdU and PDGFR-α or BrdU, GSTpi and APC were examined using a Leica SP2 laser scanning confocal microscope and 63x oil-immersion apochromatic objective. To avoid misinterpretation of multiple labeling due to potential overlap in excitation and emission spectra, sections were imaged first at 488 nm and 633 nm to visualize Alexa 488- and Alexa 633-conjugated secondary antibodies and then at 543 nm to visualize Cy3. In each section analyzed every BrdU-labeled cell within the CC was scored for co-expression of the other marker(s); a range of 150–250 cells were analyzed per animal. Analysis of co-expression was completed on-line with no processing or enhancement of the confocal images. In addition to analyses in the CC, the phenotypes of BrdU-labeled cells in the pyriform cortex (as defined above) were analyzed in three replete and three deficient rats in the Proliferation (double-labeling for BrdU and PDGFR-α) and Survival groups (double-labeling for BrdU and APC).

Statistical analyses

Except where noted, seven replete and seven deficient *dw/dw* rats were analyzed in the Proliferation group and nine replete and nine deficient animals were analyzed in the Survival group. Statistical analyses were performed using SigmaStat 3.0 statistical system software (Systat Software Inc., San Jose CA). The experimental variables related to glial turnover were compared between GH/IGF-I replete and deficient *dw/dw* rats for each sacrifice group using two-tailed t-tests. The dependent variables included: number of BrdU-labeled cells, percentage of BrdU-labeled cells co-labeled with PDGFR-α or oligodendrocyte markers, and estimated number of cells co-labeled for BrdU and PDGFR-α or oligodendrocyte markers. We made no correction for multiple t-tests since the number of comparisons was small and all were planned a priori; the threshold of significance was set at $p \leq 0.05$. All data are presented as mean ± SEM.

RESULTS

Regulation of GH/IGF-I in dw/dw rats

The regulation of IGF-I levels and of brain and body weight in the *dw/dw* rats used in this study have been described previously (Lichtenwalner et al., 2006). In brief, IGF-I levels in

plasma and cerebrospinal fluid (CSF) did not differ between *dw/+* control rats and *dw/dw* rats treated twice daily with GH (GH/IGF-I replete) but were reduced by approximately 50% in *dw/dw* rats following cessation of GH treatment. GH injections increased the growth rate of dwarf rats to match that of heterozygous controls, whereas the weight gain of untreated dwarfs was significantly lower. After GH-treated *dw/dw* rats were switched from GH to saline injections (on P98) their weight gain ceased and within three days they weighed less than their replete littermates.

The primary measure of proliferation and cell survival was the density of BrdU-labeled cells in the CC. It was necessary, therefore, to establish that there was no change in brain size or the volume of the CC analyzed that might produce a change in density that did not reflect a change in cell number. Brain weight did not differ between replete and deficient rats (1.63±0.03 vs 1.61±0.01 g, respectively) and previous analyses revealed no effect of GH/IGF deficiency on the volume of the granule cell layer or hilus in the dentate gyrus of the hippocampus (Lichtenwalner et al., 2006). In the present study, there was no effect of GH/IGF deficiency on the average area of the CC in the sections analyzed (1.55±0.04 mm^2 in replete vs 1.59±0.04 mm^2 in deficient, $p>0.5$), indicating that the size of the CC in the region selected for analysis was unchanged.

GH/IGF-I deficiency decreases proliferation of OPCs in the cerebral white matter

Counts of BrdU-labeled cells in the CC in rats in the Proliferation and Survival groups served as measures of overall cell proliferation and of survival of the newborn cohort, respectively (Figure 1 and Figure 2). Testing for effects of GH/IGF status on proliferation by comparing the density of BrdU-labeled cells in replete and deficient rats in the Proliferation group revealed that GH/IGF deficiency decreased proliferation in the CC by approximately 25% (Figure 2A, $p<0.005$, two-tailed t-test).

Counting BrdU-labeled cells that were co-labeled for PDGFR-α assessed specifically the proliferation of presumptive OPCs. The percentage of BrdU-positive cells that were PDGFR-α^+ was the same in GH/IGF-I replete and deficient rats in the Proliferation group (82±5% vs 80±4%, respectively), but the estimated number of BrdU+/ PDGFR-α^+ cells in the CC (calculated from the density of $BrdU^+$ cells and percentage that were PDGFR-α^+) was reduced approximately 25% in deficient rats (Figure 2B, C; Figure 3A–L; $p<0.02$ by two-tailed t-test).

GH/IGF-I modulates the survival of adult born oligodendrocytes in the CC

In Survival group animals the twice daily injections of GH were continued in all animals through the day of BrdU injections and then deficient animals were switched to saline injections for the remainder of the experiment. Thus, all animals in the Survival group had the same GH/IGF status at the time of BrdU labeling and BrdU labeling of all animals in the Survival group was similar to that in GH/IGF-I replete animals in the Proliferation group (but cannot be equated exactly; rats in the Proliferation group were injected with BrdU at P111 versus P98 in Survival group animals). The density of BrdU labeled cells in all rats in the Survival group was substantially lower than that in replete rats in the Proliferation group, indicating that only a fraction of adult born cells in the CC survived for four weeks. Significantly, the density of BrdU-labeled cells was almost 30% lower in GH/IGF deficient animals than in replete littermates in the Survival group ($p<0.0001$, Figure 2A, two-tailed t-test), indicating that GH/IGF deficiency decreased the survival of adult born cells in the CC.

To assess specifically the differentiation and survival of oligodendrocytes, sections from GH/IGF replete and deficient rats in the Survival group were double-labeled for BrdU and two markers of oligodendrocytes, APC and GSTpi (Figure 3M–P and Figure 4). APC and

GSTpi labeled essentially the same population of oligodendrocytes, since virtually all cells labeled for APC also were positive for GSTpi. APC^+ and/or $GSTpi^+$ cells accounted for approximately 90% of the BrdU labeled cells in Survival group animals (Figure 4A). The percentage of $BrdU^+$ cells that expressed oligodendrocyte markers did not differ between GH/IGF-I deficient and replete rats ($p>0.5$), but estimation of the number of $BrdU^+/APC^+$ and $BrdU^+/GST^+$ cells (from the counts of $BrdU^+$ cells and percentages of double-labeled cells) indicated that the number of oligodendrocytes in the $BrdU^+$ population was 30% lower in GH/IGF deficient rats than in replete littermates ($p<0.0005$ for each marker, two-tailed t-test, Figure 4B).

Oligodendrocyte turnover in gray matter

In rats of both the Proliferation and Survival groups BrdU-labeled cells were distributed throughout the brain. Counts of BrdU-labeled cells in the pyriform cortex revealed that i) the density of BrdU-labeled cells was approximately five-fold lower than in the CC, ii) the density was not appreciably lower in the Survival group than in the Proliferation group, and iii) there was no detectable difference in density between GH/IGF-I deficient and replete rats in the Proliferation or Survival group (Figure 5). Regardless of GH/IGF-I status, approximately 80% of $BrdU^+$ cells in the pyriform cortex of Proliferation group rats expressed PDGFR-α and approximately 95% of $BrdU^+$ cells in Survival group rats expressed APC. Qualitative assessment indicated that similar majorities of BrdU-labeled cells in other regions of gray matter were oligodendrocyte precursors (Proliferation group) or oligodendrocytes (Survival group).

DISCUSSION

Accumulating evidence indicates IGF-I is an important regulator of adult neurogenesis and that decreased GH/IGF-I signaling contributes to the aging-related decline in neurogenesis (Åberg et al., 2000; Lichtenwalner et al., 2001; 2006; Trejo et al., 2001; Shetty et al., 2005). This study demonstrates that circulating GH and/or IGF-I dynamically influence oligodendroglial turnover in the white matter. Two to four weeks of adult-onset deficiency of GH/IGF-I decreased proliferation of dividing OPCs in the subcortical white matter and decreased survival of newborn oligodendrocytes. Since both GH and IGF-I levels are reduced in this model it is not possible to establish the critical mediator(s) of the observed effects on glial genesis. IGF-I is viewed as the effector for GH and several studies implicate IGF-I as a regulator of remyelination in multiple sclerosis (reviewed in Chesik et al., 2007), but GH also may have direct effects in the brain. GH and IGF-I in circulation can cross the blood brain barrier (Coculescu, 1999; Armstrong et al., 2000; Pan et al., 2005); in addition, GH and IGF-I are produced (Niblock et al., 1998; Donahue et al., 2002; Sun et al., 2005) and their receptors are abundant in numerous brain areas (e.g., Bohannon et al., 1988; Lesniak et al., 1988; Lobie et al., 1993; Zhai et al., 1994; Nyberg and Burman, 1996; Guan et al., 1997). Thus, there is the potential for both endocrine and paracrine/autocrine actions of both GH and IGF-I. Analysis of a separate cohort of GH/IGF-I replete and deficient rats revealed no change in IGF-I levels in the brain, despite the significant decrease in plasma (Linville et al., 2008), suggesting that direct effects of GH and/or plasma levels of GH and/or IGF-I were responsible for the observed effects on oligodendrocyte turnover.

Regulation of Proliferation by GH/IGF-I

The BrdU injection protocol used in the present study provided a reasonable index of cell proliferation, since animals were administered BrdU over six hours and perfused 18 hours later. Among limited estimates of cell cycle kinetics for glial precursors in the adult brain, Watanabe et al. (2002) estimated a cell cycle time for oligodendrocyte precursors greater than 24 hours following a demyelinating lesion and turnover appears to be much slower in

the intact adult brain (see, for example, McCarthy and Leblond, 1988; Shi et al., 1998). The 18 hour survival period following BrdU labeling in the present study likely was insufficient for precursors to re-enter the cell cycle and give rise to another generation of daughter cells. Thus, our protocol provided a reasonable assessment of proliferation of progenitor cells in the CC.

The present results demonstrate that two weeks of GH/IGF-I deficiency is sufficient to reduce proliferation of dividing cells in the CC by 25%, suggesting dynamic regulation of progenitors in the white matter. Moreover, analysis of double labeling for BrdU and PDGFR-α revealed a GH/IGF-dependent reduction in the number of OPCs within the $BrdU^+$ population that was similar in magnitude to the decline in $BrdU^+$ cells. Thus, it appears that the deleterious effects on proliferation are specific for OPCs.

Regulation of Survival and Maturation

Our results reveal a robust effect of GH/IGF-I on survival of adult-born oligodendrocytes. Within the $BrdU^+$ cohort of newborn cells, the number that expressed markers of mature oligodendrocytes was reduced 30% by GH/IGF-I deficiency. In principle, GH/IGF deficiency could have affected only survival or also commitment of cells to the oligodendrocyte lineage, but the most parsimonious explanation for the current findings is that GH/IGF-I deficiency primarily decreased survival. If GH/IGF-I deficiency reduced commitment to the oligodendrocyte lineage, one would expect in deficient animals an increase in the percentage of $BrdU^+$ cells that did not express markers of oligodendrocytes, which was not the case. The reduction in the total number of cells in the $BrdU^+$ cohort in GH/IGF-I deficient rats was accounted for by the reduction in $APC^+/GSTpi^+$ cells. Regulation of survival of adult-born oligodendrocytes is consistent with previous evidence that IGF-I affects survival of both mature oligodendrocytes and differentiating oligodendrocytes following demyelinating lesions or other manipulations that deplete oligodendrocytes (e.g., Liu et al., 1995; Ye and D'Ercole, 1999; Mason et al., 2000; Guan et al., 2001; Kumar et al., 2007).

An effect of GH/IGF-I on migration of newborn oligodendrocytes also could have contributed to decreased turnover of oligodendrocytes in the CC of deficient rats. Regulation of oligodendrocyte migration by the GH/IGF-I system has not been directly investigated, but there is evidence that IGF-I promotes motility and migration of Schwann cells and glioblastoma cells (Guvakova, 2006; Schlenska-Lange et al., 2008). Moreover, combinations of growth factors that include IGF-I have been shown to increase oligodendrocyte migration following experimental demyelination (Espinosa-Jeffrey et al., 2006; Kumar et al., 2007).

Significance of GH/IGF-I-dependent regulation of oligodendrocyte turnover

We have demonstrated that plasma levels of GH/IGF-I influence adult neurogenesis (Lichtenwalner et al., 2001; 2006) and glial turnover in the white matter (current study). It is reasonable to suspect that such modulatory influences of the GH/IGF-I axis on cell turnover contribute to age-related changes in cognitive ability (e.g., Barnes, 1979; Churchill et al., 2002) and the ability of GH and IGF-I to reverse cognitive impairment associated with aging or disease (e.g., Markowska et al., 1998; Thornton et al., 2000; Lupien et al., 2003; Ramsey et al., 2004). The functional impact of changes in neuronal turnover likely is limited, since adult neurogenesis is restricted to the dentate gyrus of the hippocampus and the subventricular zone/rostral migratory stream/olfactory bulb system. In contrast, influences on the regulation of glial genesis may be widespread, since there is ongoing turnover of oligodendrocytes throughout the brain, with actively dividing OPCs in both white- and gray matter (Reynolds and Hardy, 1997; Levison et al., 1999; Gensert and Goldman, 2001;

Levine et al., 2001; Liu and Rao, 2004; Polito and Reynolds, 2005). Consistent with those previous reports, qualitative assessment of brain sections from rats in the Survival group in this study revealed $BrdU^+/APC^+/GSTpi^+$ cells in the cerebral cortex. In the present study GH/IGF-I deficiency did not have a demonstrable effect on oligodendrocyte turnover in pyriform cortex, suggesting that regulation by the GH/IGF-I axis may be specific to white matter, but the approximately five-fold lower density of proliferating cells in gray matter compared to white matter limits the ability to detect changes in the former.

There has been little assessment of the significance of ongoing oligodendrocyte replacement for normal brain function and little analysis of aging-related changes in basal turnover of oligodendrocytes. Information on oligodendrocyte turnover in normal adults comes primarily from control animals in studies of remyelination in models of demyelinating disorders. Significantly, many manipulations used to reduce adult neurogenesis and probe its role in cognitive function (e.g., irradiation - Meshi et al., 2006; Saxe et al., 2006, or mitotic inhibitors - Shors et al., 2001; 2002) also affect glial proliferation. Thus, the suppression of glial turnover could contribute to cognitive changes interpreted primarily in the context of changes in neurogenesis.

It is not clear whether the maintenance of OPCs and oligodendrocyte turnover in the adult brain serves normal function or only provides a rapidly recruitable population of cells for myelin repair following damage. Proliferation of oligodendrocyte precursors and recruitment of new, myelinating oligodendrocytes from immature precursors contribute to myelin repair following demyelinating lesions. Following demyelination, proliferation of OPCs and commitment, differentiation and survival of adult-born oligodendrocytes all appear to be targets of regulation by inflammatory cytokines and growth factors (see Armstrong, 2007 for recent review). The GH/IGF-I system appears to play a particularly critical role in myelin repair. IGF expression is induced in multiple models of demyelination and is increased during remyelination (e.g., Liu et al., 1994; Hinks and Franklin, 1999; Fushimi and Sharabe, 2004). Treatment with IGF-I or overexpression of IGF-I in transgenic mice inhibits oligodendrocyte death during demyelination and/or enhances remyelination following demyelinating lesions (e.g., McMorris and McKinnon, 1996; Mason et al., 2000; Kumar et al., 2007). Studies of lysolecithin-induced demyelination in the spinal cord of young adult and old adult rats suggest that slower and less effective increases in IGF-I expression contribute to an aging-related decrease in the efficacy of remyelination (Hinks and Franklin, 2000), which appears to be attributable to impairments in both OPC recruitment and differentiation of newborn oligodendrocytes (Sim et al, 2002; Chari et al., 2003).

Although IGF-I may have potential in the treatment of demyelinating disorders, IGF-I has not been protective in all experimental studies of demyelination (Canella et al., 2000; O'Leary et al., 2002; Genoud et al., 2005) and a pilot study of recombinant IGF-I in multiple sclerosis patients failed to show benefits (Frank et al., 2002). Given the potential of IGF to promote tumorigenesis and the complexity of the IGF system, with multiple binding proteins regulating activity (discussed in Chesik et al., 2007; 2008), translating evidence from experimental studies that IGF influences remyelination to clinical practice is a significant challenge. Evidence from assays of plasma and cerebrospinal fluid (CSF) from MS patients that revealed no change in IGF-I levels but reduced GH in CSF (Poljakovic et al., 2006; Hosback et al., 2007) may support more investigation of direct effects of GH on oligodendrocytes and myelination, independent of any actions mediated by IGF-I.

Conclusion

There is accumulating evidence that the GH/IGF-I system is a critical regulator of the genesis and turnover of oligodendrocytes. Effects of the GH/IGF-I axis are not limited to

development, but rather may influence replacement of oligodendrocytes in the normal adult brain and following demyelinating lesions. Aging-related changes in GH/IGF-I signaling may contribute to aging-related changes in the ability of the CNS to repair damage and to cognitive changes associated with normal aging. The relatively rapid and dynamic regulation of OPCs and developing oligodendrocytes demonstrated in the present study suggests that short-term manipulation of GH and/or IGF-I, if appropriately timed, might have benefits in the treatment of demyelinating disorders.

Acknowledgments

This work was supported by National Institutes of Health Grant PO1 AG11370 (D.R.R., W.E.S.) and by WFUSM Venture Funds (D.R.R.). The authors wish to thank Rhonda Ingram for technical assistance.

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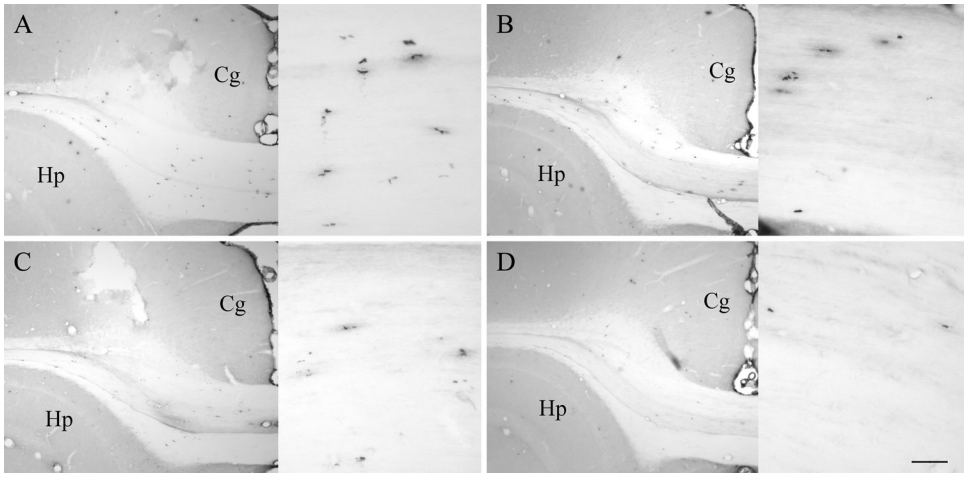

Figure 1. S-phase labeling with BrdU

IHC labeling for BrdU identified newborn cells in the subcortical white matter at 18 hours (Proliferation group, A and B) and four weeks (Survival group, C and D) after BrdU injection in GH/IGF-I replete (A and C) and deficient (B and D) rats. The cingulate cortex (Cg) and hippocampus (Hp) are identified in each section for orientation. Inset: medial CC at higher magnification. Scale bar = 250 μm, 50 μm (inset).

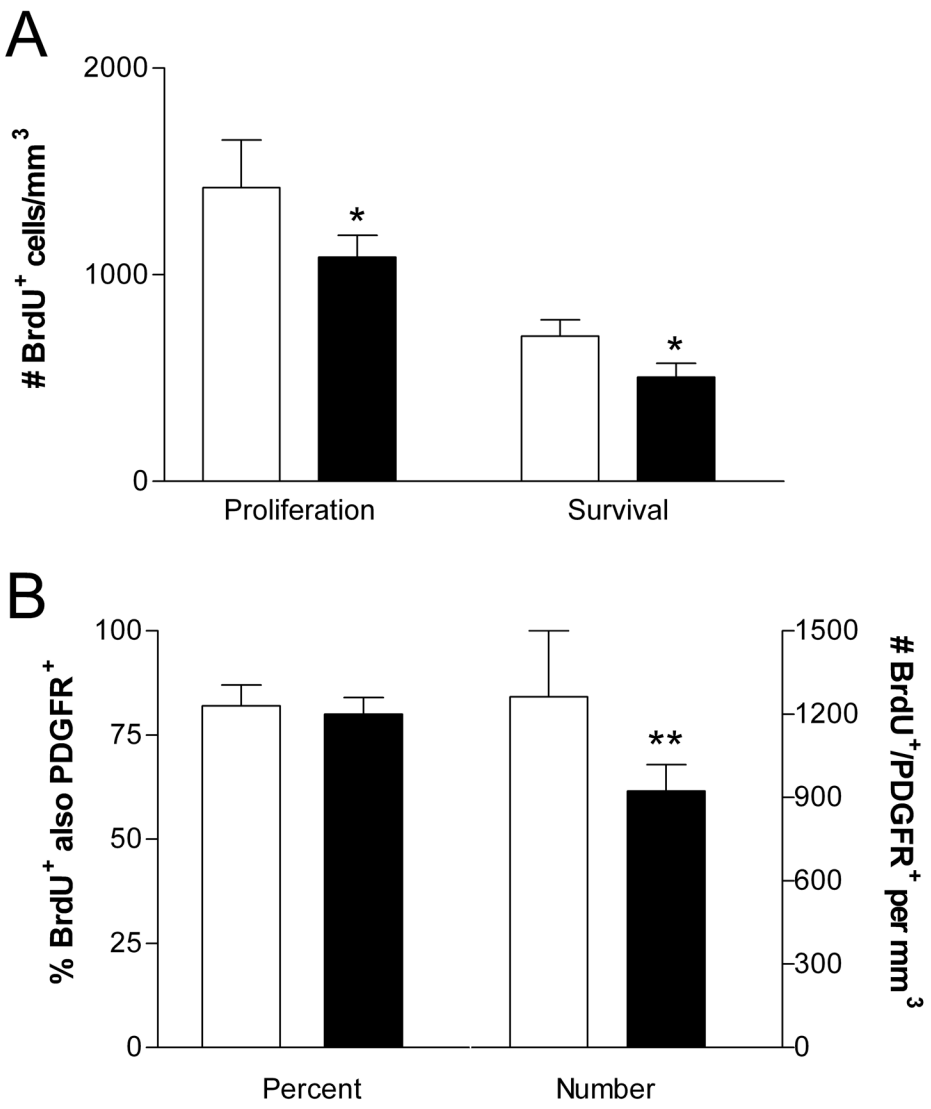

Figure 2. Counts of proliferating cells and OPCs

The density of $BrdU^+$ cells (A) is shown for GH/IGF-I replete (open bars) and deficient rats (filled bars) in the Proliferation and Survival groups. $BrdU^+$ cells were reduced in deficient compared to replete rats in both groups, indicating decreased proliferation of progenitor cells and decreased survival of newborn cells. In the Proliferation group (B) the percentage of $BrdU^+$ cells that were PDGFR-α^+ was not affected by GH/IGF-I deficiency, but the estimated density of $BrdU^+$/ PDGFR-α^+ cells (#/mm^3) was reduced. All values mean±sd; *$p<0.005$ and **$p<0.02$ versus GH/IGF-I replete littermates.

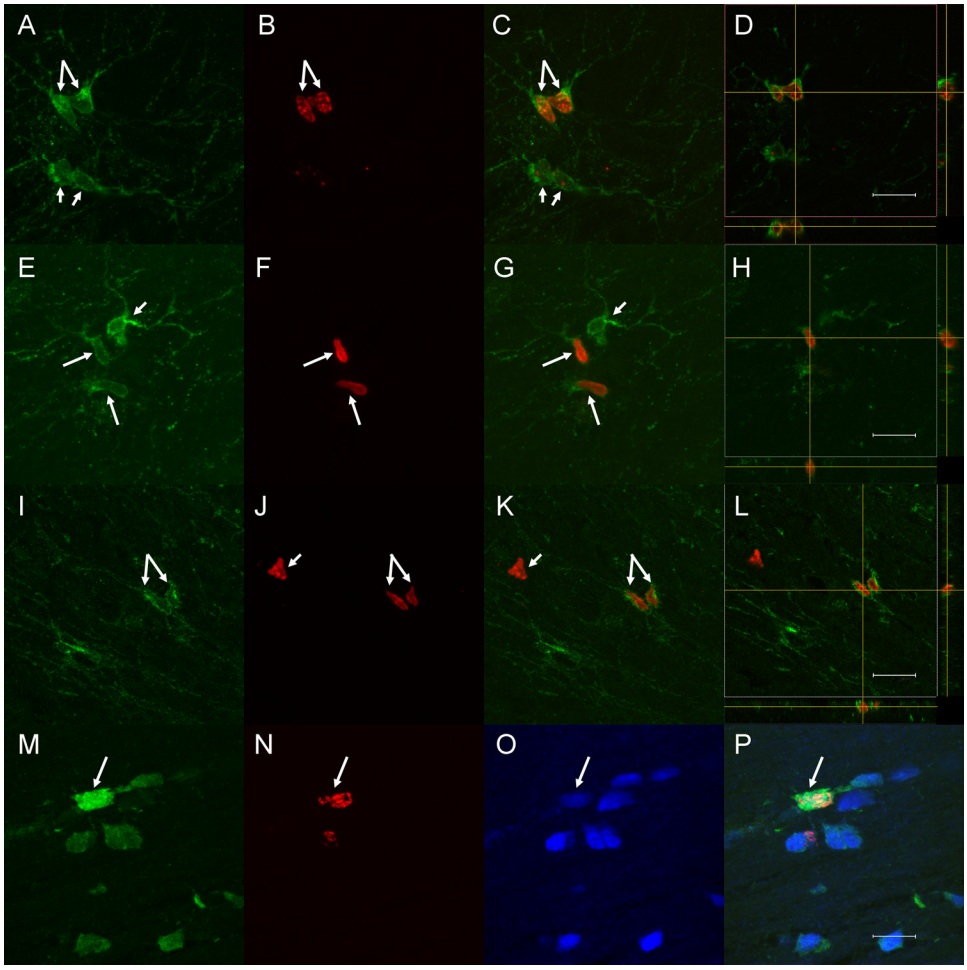

Figure 3. Immunofluorescent labeling for BrdU, PDGFR-α, and oligodendrocyte markers

Double labeling for BrdU (red) and PDGFR-α (green) in Proliferation group rats (A–L) revealed numerous double labeled cells (long arrows), some adjacent pairs that suggested recent division (e.g., C and K). PDGFR-α^+ cells that were not $BrdU^+$ (short arrows in A–G) and BrdU+ cells that were not PDGFR-α^+ (short arrow in J–K) also were evident. Three-dimensional imaging and orthogonal views (D, H and L) confirmed that $BrdU^+$ nuclei were located within PDGFR-α^+ cells, not adjacent to them. Triple labeling for BrdU (red), APC (green) and GSTpi (blue) in sections from rats in the Survival group (M–P) demonstrated triple labeled cells (long arrow, merged image in P), evidence of ongoing addition of oligodendrocytes in adult rats. Scale bars = 12 μm (A–H; M–P), 16 μm (I–L).

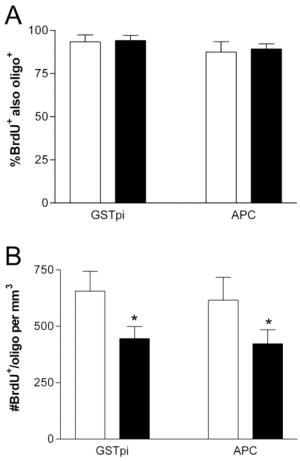

Figure 4. Counts of newborn oligodendrocytes

The percentage of $BrdU^+$ cells that were labeled with oligodendrocyte markers (A) and the estimated density of $BrdU^+$ cells that were $GSTpi^+$ and/or APC^+ (B) are shown for GH/IGF-I replete (open bars) and deficient (filled bars) rats in the Survival group. GH/IGF-I deficiency significantly reduced the number of newborn (BrdU+) cells that were identified as oligodendrocytes. All values mean±sd; *$p<0.0005$ versus GH/IGF-I replete littermates.

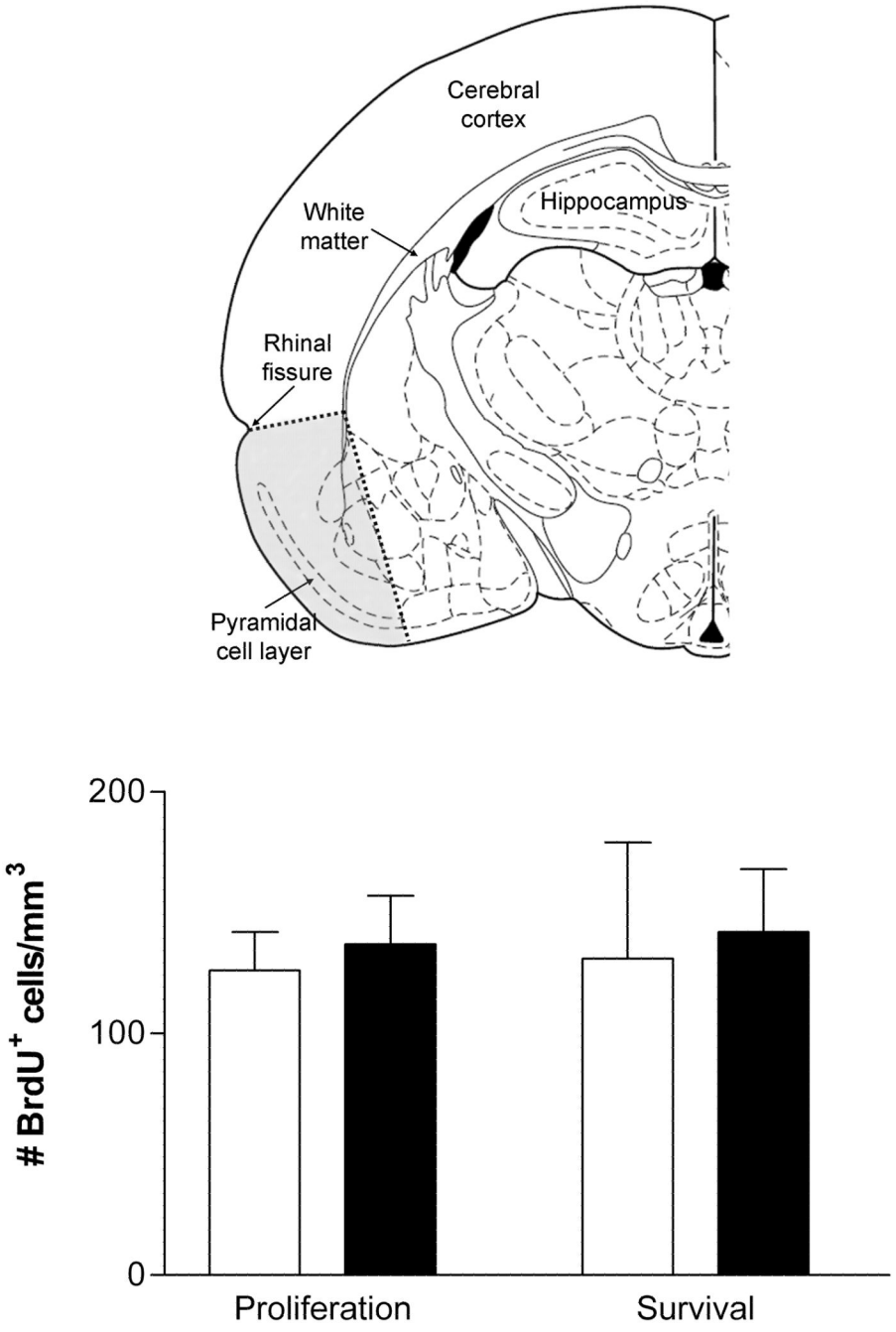

Figure 5. Counts of proliferating and newborn cells in the pyriform cortex

Oligodendrocyte turnover in the cerebral cortex was quantified in pyriform cortex (shaded area above) as defined in the text. The density of $BrdU^+$ cells, shown for GH/IGF-I replete (open bars) and deficient rats (filled bars) in the Proliferation and Survival groups, was similar at the two time points and did not differ between GH/IGF-I deficient and replete rats. All values mean±sd.