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Adult sulfatide null mice maintain an increased number of oligodendrocytes

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Abstract

The galactolipids galactocerebroside and sulfatide have been implicated in oligodendrocyte development and myelin formation. Much of the evidence for these galactolipid functions has been derived from antibody and chemical perturbation of cultured oligodendrocytes. Recently, we have observed abundant, unstable myelin and an increased number of oligodendrocytes in mice incapable of synthesizing the myelin galactolipids galactocerebroside and sulfatide. We have also reported that mice lacking sulfatide but that synthesize normal levels of galactocerebroside generate myelin with unstable paranodes while Hirahara et al. (2004) have shown an enhanced population of oligodendrocytes in the forebrain, medulla and cerebellum in immature sulfatide null mice. Here, we demonstrate that an increase in the number of oligodendrocytes in sulfatide null mice is not transient but is maintained through, at least, 7 months of age. Moreover, we demonstrate that the enhanced oligodendrocyte population results from, at least in part, increased cell survival. Finally, sulfatide null oligodendrocytes exhibit decreased morphological complexity, a feature which may relate to increased oligodendrocyte survival.

Keywords

sulfatide; oligodendrocyte; development; cell survival

Introduction

The combination of early appearance and abundance of the galactolipids, galactocerebroside and sulfatide, has resulted in intense interest in the significance of these lipids in oligodendrocyte development and myelin sheath formation and maintenance (Pfeiffer et al., 1993; Taylor et al., 2004). Much of the initial work designed to elucidate the roles of the galactolipids was conducted *in vitro* using a variety of immunological and chemical perturbations (reviewed in Dupree and Popko, 1999). For example, Dubois-Dalcq et al. (1970) reported that the addition of cerebroside anti-sera inhibited myelin formation in culture. These findings were subsequently confirmed by several groups who demonstrated that antibody perturbation not only inhibited myelin formation (Ranscht et al., 1987; Owens

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and Bunge, 1990) but also compromised myelin integrity *in vitro* (Fry et al., 1974; Saito et al., 1986; Saida et al. 1979; Roth et al., 1985, Bansal and Pfeiffer, 1994) and *in vivo* (Sergott et al., 1986; Rosenbluth et al., 1994; 1995).

In addition to myelin formation and maintenance, Steve Pfeiffer and colleagues published a series of seminal articles that provided the foundation for our understanding of the roles that the galactolipids play in oligodendrocyte development. In 1989, Bansal and Pfeiffer demonstrated that the addition of an antibody that recognizes both galactocerebroside and sulfatide (Ranscht monoclonal antibody, Ranscht et al., 1982) inhibited oligodendrocyte progenitors from terminal differentiation; however, antibody removal resulted in continued differentiation and morphological changes consistent with oligodendrocyte maturation. Although these results were exciting, this antibody, which recognized both galactocerebroside and sulfatide, did not allow a distinction between the function of these two closely related lipids. Thus, Pfeiffer and colleagues conducted several studies aimed to perturb specifically the function of galactocerebroside or sulfatide (Bansal et al., 1988; Bansal et al., 1989; Bansal and Pfeiffer, 1989). The culmination of these works suggests that sulfatide is the primary galactolipid regulatory molecule in oligodendrocyte development.

More recently, genetically engineered mice deficient in galactolipid synthesis (Bosio et al., 1996; Coetzee et al., 1996; Honke et al., 2002) have been employed to further our understanding of the role that these lipids play in oligodendrocyte development. In mice lacking either sulfatide or both sulfatide and galactocerebroside, oligodendrocytes express mature myelin markers and form abundant myelin sheaths (Coetzee et al., 1996; Honke et al., 2002, Marcus et al., 2006). Although oligodendrocytes in these mice terminally differentiate and form myelin, the regulatory mechanisms that control oligodendrocyte numbers are compromised as both mutants exhibit enhanced numbers of terminally differentiated oligodendrocytes in young mice (Marcus et al., 2000; Hirahara et al., 2004). Presently, it is not clear why the oligodendrocyte populations in these mice are increased or whether these increased populations are maintained throughout life. Here, we have explored these questions using the sulfatide null mice. Our results show that the increased oligodendrocyte population is indeed maintained in adult sulfatide null mice. We also report increased proliferation in the central nervous system of young mutant mice, an increase that is at least partially related to cells of the oligodendrocytic lineage. In addition, significantly fewer sulfatide null oligodendrocyte progenitors undergo normal, postnatal apoptosis suggesting that increased survival plays a role in establishing and maintaining the enhanced oligodendrocyte population. Finally, we provide ultrastructural evidence that the sulfatide null oligodendrocytes extend fewer myelin forming processes, a feature that may allow more oligodendrocytes to establish axonal contact resulting in an increase in the number of oligodendrocytes that survive developmental pruning. Taken together, these findings strongly substantiate the work of Dr. Pfeiffer and his colleagues by demonstrating a role for sulfatide in proper oligodendrocyte development *in vivo*.

Materials and Methods

Animals

All animals used in this study were generated and housed in the Virginia Commonwealth University Division of Animal Resources. Mice heterozygous for the gene that encodes ceramide sulfotransferase (CST) were mated and offspring were genotyped as previously described (Honke et al., 2002) with the following slight modifications. Genotypes of the CST mice were determined by genotyping with primers specific to the CST gene (CSTFL: 5′-CTA TTG GAC AAC TAC CCA CTA CCA CCT GC-3′ and CSTRL: 5′-GCA CTT ATG TCC GTG TGA GAG TGT CAG GTC-3′) and to the neo cassette (CSTNEOF: 5′-CAT TCG ACC ACC AAG CGA AAC ATC G-3′ and CSTNEOR: 5′-GCA CGA GGA AGC GGT CAG CCC AAT-3′). PCR cycles were as follows: 95°C for 5 minutes, [95°C for 10 seconds, 60°C for 10 seconds, 68°C for 20 seconds] for a total of 30 cycles with Klentaq DV Ready Mix (Sigma Chemical, St. Louis, MO). Wild type (WT) and sulfatide null mice yield only a single PCR product of 548 base pairs and 332 base pairs, respectively. Heterozygous mice yield both products.

Immunocytochemistry

WT and littermate sulfatide null mice 15 days, 30 days and 7 months of age were perfused with 4% paraformaldehyde in 0.1M Millonigs phosphate buffer (pH 7.3); brains and spinal cords were harvested, cryoprotected, frozen, transversely sectioned and immunolabeled as previously described (Dupree et al., 1999; Dupree et al., 2005). Primary antibodies required for these studies included CC1, anti-olig2, anti-glial fibrillary acidic protein (GFAP) and anti-bromodeoxyuridine. The CC1 antibody (CalBiochem, Cambridge, MA; mouse monoclonal, 1:100), recognizes mature oligodendrocyte cell bodies without labeling the myelin sheath (Bhat et al., 1996). We and others have successfully used the CC1 marker to label oligodendrocytes in developing, mature and remyelinating systems *in vivo* (Fuss et al., 2000; Dupree et al., 2005; Messersmith et al., 2000; Murtrie et al., 2005; Sohn et al., 2006; Vana et al., 2007). The olig2 antibody (rabbit polyclonal, 1:10,000), which was kindly provided by Drs. John Alberta and Chuck Stiles (Dana-Farber Cancer Institute, Boston, MA), recognizes oligodendrocyte progenitor cells and mature oligodendrocytes (CNPase positive and PLP positive) (Ligon et al., 2004). The GFAP antibody was obtained from Thermo/Fisher Scientific (Pittsburgh, PA; rabbit polyclonal, 1:200). The anti-bromodeoxyuridine (B-D Biosciences, San Jose CA; 1:200) was directly conjugated to FITC; all other primary antibodies were visualized by indirect labeling with a fluorescently tagged secondary antibody. All immunofluorescently labeled sections were imaged using either a Leica TCS-SP2 AOBS confocal laser scanning microscope (inverted) with a spectrophotometer scan head or a Nikon Eclipse E800M microscope equipped with Diagnostic Instruments Spot RT Camera (Diagnostic Instruments Inc., Sterling Heights, MI).

Cell type quantitation

All quantitative analyses were limited to the ventral columns of the cervical spinal cord. Ventral columns were delineated centrally by neuronal cell bodies of the grey matter and by exit points of the ventral roots. To quantitatively compare mature astrocyte and

oligodendrocyte populations, four 1600 × 1200 pixel images per spinal cord (two images per side) were collected using the Nikon Eclipse E800M microscope with a Plan Fluor 40×/0.75 NA objective lens. For this objective lens, the image depth of field is approximately 1 μm thick. Based on the pixel parameters and objective used for image collection, each pixel equaled 0.183 μm per side hence the microscopic field represented 0.064 mm^2.

To compare mature astrocyte and oligodendrocyte populations, the numbers of DAPI+/GFAP+ and CC1+ cells per microscopic field, respectively, were determined for each genotype at 15 days and 30 days of age. In addition the number of CC1+ cells was also determined at 7 months of age. The relative number of oligodendrocyte progenitor cells (OPCs) was determined by employing an antibody directed against olig2, a transcription factor expressed by cells in the oligodendrocyte lineage (Lu et al., 2000; Zhou et al., 2000) that is used to identify OPCs (Pernet et al., 2008). Since olig2 is also present in differentiated oligodendrocytes (Ligon et al., 2004; Gokhan et al., 2005), we triple labeled spinal cord sections with the olig2 antibody, the CC1 antibody and DAPI. DAPI+/olig2+/CC1+ were considered mature oligodendrocytes and were not included in the OPC counts; DAPI+/olig2+/CC1- cells were identified as OPCs. For both mature oligodendrocyte and OPC counts, at least four littermate pairs were analyzed at 15 days, 30 days and 7 months of age and three sections per animal were analyzed per antibody combination. To account for variability among litters, all data are presented as mean number of cells per microscopic field expressed as a percent of littermate WT ± percent standard deviation.

Apoptosis analysis-Terminal uridine deoxynucleotidyl transferase dUTP nick end (TUNEL) labeling

Transverse cryosections (10 μm thick) of cervical spinal cords (prepared as described above) from 15 day old WT and sulfatide null mice were immersed in -20°C acetone and further permeabilized by incubation in 0.1% Triton-X-100 in 0.1% sodium citrate. The sections were then incubated in the TUNEL reagent as described by the manufacturer (Roche, Boulder, CO; *in situ* Cell Death Detection Kit) and labeled cells were visualized as described above. For quantification of TUNEL labeling, at least three 10 μm frozen transverse spinal cord sections per mouse from six sulfatide null and eight littermate WT animals from three litters were triple labeled with DAPI, the olig2 antibody and the TUNEL reagent. Similar to the OPC data, counts for the TUNEL analysis are expressed as mean cell number per field expressed as a percent of littermate WTs ± percent standard deviation.

Proliferative analysis- Bromodeoxyuridine studies

BrdU (Sigma Chemical) was prepared as per the manufacturer's directions. Briefly, 20 mg/mL of BrdU was prepared in 0.9% saline containing 0.007N sodium hydroxide. 15 day old WT and sulfatide null littermates were intraperitoneally injected with 200 mg/kg body weight using the 20 mg/mL stock BrdU solution. Two hours after injection, the animals were perfused and spinal cord and brain tissues were processed for immunohistochemistry as described with the addition of a 30 minute incubation in 3N HCl. Similar to the TUNEL analysis, a minimum of three 10 μm frozen transverse spinal cord sections from a total of five sulfatide null mice and seven littermate WT animals from three litters were analyzed. The data are presented as mean percent of WT ± percent of standard deviation.

Light and electron microscopy

Spinal cord tissue from WT and sulfatide null animals was prepared as previously described with slight modifications (Dupree et al., 1998; Marcus et al., 2006). Animals were perfused with 0.1 M Millonigs phosphate buffer (pH 7.3) containing 4% paraformaldehyde and 5% glutaraldehdye. Samples were post fixed in 1% osmium tetroxide, dehydrated in serial dilutions of ethanol and embedded in PolyBed 812 resin (PolySciences, Warrington, PA). Thick (1 μm) and ultrathin (90 nm) sections were stained with toluidine blue or uranyl acetate and lead citrate, respectively. Thick sections were qualitatively assessed using a Nikon Eclipse E800 upright compound microscope, equipped with a Spot camera, using an oil immersion Plan Fluor 100×/1.3 N.A. objective lens. The ultrathin sections were analyzed using a JEOL 1230 transmission electron microscope equipped with a Gatan UltraScan CCD camera, which is housed in the Virginia Commonwealth University Department of Anatomy and Neurobiology Microscopy Facility.

Quantification of oligodendrocyte processes

Cells in the WT and mutant spinal cord ventral columns not containing either intermediate filaments or glycogen granules, presumed to be oligodendrocytes, were imaged at 3000×. The number of primary oligodendrocyte processes, defined as processes that branched directly from the cell body (Figure 1a), was determined for each soma. Analysis was conducted on presumptive oligodendrocytes from 2 and 4 day old mice as the processes are substantially more reliably delineated than in spinal cords from adult animals. The data are presented as the mean number of primary processes per cell ± standard deviation.

Statistical analyses

All data were statistically compared using 2-tailed t-tests. Statistical significance was accepted with a p value of 0.05 or less.

Results

Adult sulfatide null mice maintain a larger population of mature oligodendrocytes than their WT littermates

Consistent with our previous analysis of mice that lack both galactocerebroside and sulfatide (Marcus et al., 2000), qualitative assessment of the thick sections indicated that young sulfatide null mice, like WT littermates, exhibit numerous putative oligodendrocytes in the ventral column of the spinal cord (Figure 1a). Although the cellularity is dramatically reduced in both WT and mutant animals by 7 months of age, the sulfatide null mice display dramatically more cells than littermate controls (Figure 1 b and c). From these 1 μm sections, we were unable to determine whether the increased cellularity in the null mice represents an increase in cell number or if the cells are merely more apparent resulting from enlarged cell bodies and increased interaxonal spacing. Although many of the cells display morphologic characteristics consistent with oligodendrocytes (Figure 1c, inset), the identity of the cells can not be determined from the 1 μm sections. Therefore, we employed immunocytochemical analyses to determine cell identity and to compare quantitatively the numbers of these cells in the WT and mutant mice.

Since sulfatide is expressed in both astrocytes and oligodendrocytes (Pernber et al., 2002; Molander-Melin et al., 2004), these cell types are strong candidates for perturbation with sulfatide deficits. DAPI+/GFAP+ cells were abundant in the ventral columns of both the 15 day and 30 day old WT and sulfatide null mice; however, the number of DAPI+/GFAP+ cells did not differ between the 2 genotypes at either age (data not shown), indicating that the astrocyte population is not altered in the sulfatide null mice. In contrast to the astrocytic analysis, the number of CC1+ cells was significantly increased at 15 days of age, yielding a population that was 123.4% ±16.6% of the WT population (85.0±9.9 cells per field compared to 68.9±5.2 cells per field in the WT mice ($p=0.002$)) (Figure 2). Similarly, a significant increase was also observed in 30 day old sulfatide null mice which exhibited a population that was 152.5% ± 10.9% of the WT population of mature oligodendrocytes (100.2±6.3 CC1+ cells per field compared to 65.7±8.1 CC1+ cells per field in WT littermates ($p=2.6\times10^{-8}$). As the mature oligodendrocyte population was enhanced at both 15 and 30 days of age, we analyzed the oligodendrocyte population in 7 month old animals to determine whether the enhanced number of oligodendrocytes was maintained in adult animals. Interestingly, by 7 months of age, the actual number of CC1+ cells per field in both the WT and sulfatide null mice was reduced as compared to the 30 day old mice (79.2±8.4 CC1+ cells per field compared to 44.7±5.3 CC1+ cells per field in 7 month old mutant and WT animals, respectively). However, the percent difference between the mutants and the WT littermates at this advanced age was greater (CC1 population in the mutant mice was 177.2% of the WT population) and remained significantly enhanced in the sulfatide null mice ($p=1.2\times10^{-6}$).

To determine whether the difference in cell density (number of cells per microscopic field) reflected an actual increase in oligodendrocyte number, we utilized 1 micron thick sections to measure and compare the area of the ventral column white matter tracts at 15 days, 30 days and 7 months of age. No difference in the size of the ventral column white matter tracts was observed between the WT and sulfatide null mice at 15 days. Thus, we conclude that by 15 days of age, the sulfatide null mice maintain significantly more mature oligodendrocytes than littermate WT animals.

Although the area of the white matter tracts was not different between the null and WT mice at 15 days of age, the ventral columns in both the 30 day and 7 month old sulfatide null mice were 15% smaller than the ventral columns of the age matched WT mice. To determine whether tighter packing of an equivalent number of cells resulted in the increased densities, we adjusted the CC1 counts accordingly. The adjusted densities of CC1+ cells in the 30 days and 7 month old sulfatide null mice were 80.0±11.1 and 67.2±11.7, respectively and both adjusted densities remained significantly greater than their age matched WT counterparts (65.7±8.1 CC1+ cells per field, $p=6.3\times10^{-8}$ for 30 day old mice; 44.7±5.3 CC1+ cells per field, $p=5.9\times10^{-5}$ for 7 month old mice). It is important to note that although the ventral white matter regions are smaller in the 30 day and 7 month old sulfatide null mice, no axonal abnormalities including axonal deterioration or loss were observed until 7 months of age (Marcus et al., 2006). Even by 7 months of age, axonal loss was modest. Thus, we predict that the reduced white matter areas in the sulfatide null mice result from the significantly thinner myelin sheaths and not a reduction in the number of axons.

Sulfatide null mice exhibit increased cell proliferation

To identify the mechanism responsible for the increased oligodendrocyte population in the sulfatide null mice, we assessed the extent of cellular proliferation via BrdU labeling. Proliferation in WT and sulfatide null mice was compared by quantifying the number of DAPI+/BrdU+ cells in the white matter of the cervical spinal cord. Sulfatide null mice displayed a significant increase in the number of proliferative cells (165.9±28.6%, p=0.002) in the spinal cord white matter at PND 15 (Figure 3). Despite our best efforts, we were unable to determine the percentage of BrdU+ cells that were of the oligodendrocyte lineage as the antigen retrieval method required for labeling of incorporated BrdU apparently destroys the immunoreactivity with antibodies directed against oligodendrocyte lineage markers, including olig2, NG2, and CC1 (data not shown). Therefore, we conclude that the ventral columns of the sulfatide null mice exhibited significantly more proliferative activity than comparably aged WT spinal cord ventral columns; however, the identity of these proliferative cells remains unclear.

Olig2+ cells are more abundant in sulfatide null mice

Although our attempts to double label proliferative cells with oligodendrocytic markers were not successful, we reasoned that increased proliferation of oligodendrocyte lineage cells would result in an increased OPC population. Hence, we quantified the relative number of OPCs as identified by olig2 immunoreactivity in WT and sulfatide null mice at 15 days of age (Figure 4A-C). It should be noted that olig2 is expressed in motor neurons and astrocytes (Masahira et al., 2006). As our analysis is limited to white matter tracts, neuronal cell body labeling with the olig2 antibody does not present a problem. Also, only a subset of astrocytes express olig2 (Masahira et al., 2006) and since the number of GFAP+ cells is not altered in the sulfatide null mice, it is unlikely that any differences observed with olig2 labeling is related to astrocytic lineage cells. Additionally, single labeling with the olig2 antibody has been successfully used as an OPC marker (Nait-Oumesmar et al., 2007; Pernet et al., 2008).

With this caveat in mind, the sulfatide null mice revealed significantly more olig2+ cells (129.6%±8.3%; p=0.007) then the WT mice at 15 days of age. Since olig2 is also expressed by myelinating oligodendrocytes (Ligon et al., 2004), we triple labeled spinal cord sections with DAPI and the olig2 and CC1 antibodies in order to distinguish between OPCs and olig2+ mature oligodendrocytes. Based on the criterion that DAPI+/olig2+/CC1- cells are OPCs, the sulfatide null mice contained significantly more OPCs (170.1±62.1% of WT OPCs per microscopic field; p=0.038) than their WT littermates (Figure 4D).

Fewer oligodendrocytes undergo apoptosis in the sulfatide null mice

Based on the above analysis, OPC proliferation may play a role in initially establishing an enhanced population of oligodendrocyte lineage cells. However, Barres et al. (1992) reported that an excess of oligodendrocyte progenitors enter the developing white matter tracts and these “extra” OPCs are pruned through apoptosis. According to the axon-contact-mediated-survival paradigm (Barres et al., 1992; 1993a and b), oligodendrocytes that fail to establish sufficient axonal interactions undergo apoptosis resulting in the survival of the precise number of myelinating cells required for adequate myelin formation. AS reports

have shown that 20%-50% of the OPCs that initially enter CNS white matter regions do not survive (Barres et al., 1992; Trapp et al., 1997), the production of “extra” oligodendrocytes is not sufficient to maintain an increased population of mature oligodendrocytes. Therefore, we reasoned that increased proliferative activity in the sulfatide null mutants must be accompanied by enhanced oligodendrocyte survival.

To test this hypothesis, we performed a TUNEL assay and subsequently stained spinal cord sections with DAPI. The number of DAPI+/TUNEL+ cells in the sulfatide null mice was 76.3%±15.7% of WT. Thus, at 15 days of age, the sulfatide null mice exhibited significantly fewer apoptotic cells than their WT littermates ($p=0.01$). To compare the relative number of oligodendrocytes that are TUNEL+, we triple labeled spinal cord sections with DAPI, the olig2 antibody and the TUNEL reagent. The sulfatide null mice revealed significantly fewer TUNEL+/olig2+/DAPI+ cells than littermate WT mice (Figure 5) (36.2% fewer triple labeled cells; $p=0.02$). Thus, in the absence of sulfatide significantly fewer oligodendrocyte lineage cells exhibited apoptotic activity providing a plausible explanation for the enhanced oligodendrocyte population.

Sulfatide null oligodendrocytes extend fewer processes *in vivo*

As proposed by Barres et al. (1992; 1993a and b) and Trapp et al. (1997), immature oligodendrocytes avoid developmental apoptosis by establishing axonal contact. The competition among these oligodendrocytes plays an important role in determining the final number of myelin forming cells maintained in the adult CNS. Thus, the number of processes extended per cell, which is presumably related to the number of axonal contacts that an oligodendrocyte can establish, may be critical in finalizing the number of oligodendrocytes in the mature CNS. Since several studies have reported that sulfatide plays a role in regulating oligodendrocyte complexity with regard to process formation (Dyer et al., 1990; 1991, Bansal et al., 1988; Boggs and Wang, 2001), we proposed that the sulfatide null oligodendrocytes would extend fewer processes, thereby resulting in a significant increase in the number of immature oligodendrocytes capable of establishing axonal contact.

To determine whether the lack of sulfatide alters oligodendrocyte morphology, we employed electron microscopic analysis to compare the relative number of primary processes extended by 2 day old WT and sulfatide null oligodendrocytes *in vivo*. We determined that oligodendrocytes from the sulfatide null mice extended significantly fewer primary processes than oligodendrocytes from littermate WT mice (2.3±1.0 primary processes per mutant oligodendrocyte ($n=89$) versus 3.4±1.2 primary processes per WT oligodendrocyte ($n=91$); $p= 3.5 \times 10^{-4}$) (Figure 6a and b). Thus, as early as 2 days of age the sulfatide null oligodendrocytes extended approximately 33% fewer primary processes. By plotting a frequency distribution graph, we observed a dramatic leftward shift in the number of primary processes produced by the sulfatide null oligodendrocytes as compared to WT (Figure 6c).

To check the accuracy of these findings, we also analyzed oligodendrocytes from 4 day old mice. The data generated from the analysis of the 4 day old mice confirmed our analysis of the 2 day old mice (oligodendrocytes from the WT mice extending 2.8±1.0 primary processes per cell compared to 2.3±1.0 primary processes per sulfatide null cell ($p=0.05$)).

By combining our findings from 2 and 4 day old animals, 353 putative oligodendrocytes from five sulfatide null and six WT mice were imaged and blindly assessed for primary process formation. Thus, our findings from both the 2 and 4 day old mice indicate that sulfatide null oligodendrocytes extend significantly fewer primary processes.

Discussion

Myelin galactolipids are one of the earliest markers of terminal differentiation for oligodendrocyte lineage cells (reviewed by Pfeiffer et al., 1993). Although expressed by other cell types (Molander-Melin et al., 2004; Pernber et al., 2002), these lipids are highly enriched in oligodendrocyte membranes including the myelin sheath (Norton and Cammer, 1984). As a consequence of this early and abundant appearance, these lipids have been closely analyzed for possible roles in oligodendrocyte maturation and myelin formation (reviewed by Dupree and Popko, 1999). Here, we show that sulfatide is required for the proper regulation of the oligodendrocyte population *in vivo.* Using mice that lack the capacity to synthesize sulfatide, yet maintain normal levels of galactocerebroside (Honke et al., 2002), we show that the absence of sulfatide results in a permanent increase in the number of mature oligodendrocytes. We also provide evidence that this enhanced oligodendrocyte population in the absence of sulfatide results from increased cell survival during development. Increases in oligodendrocyte survival may be in part related to fewer processes extending from sulfatide null oligodendrocytes resulting in a need for more oligodendrocytes to contact and myelinate axons.

Enhanced number of oligodendrocytes results from increased survival

It has been established that neonatal mice lacking galactolipids contain significantly more oligodendrocytes than their WT littermates (Hirahara et al., 2004; Marcus et al., 2000); thus, we concentrated our efforts on the oligodendrocyte populations in adolescent and adult mutant animals. Our findings clearly demonstrate that the increased population of oligodendrocytes is not a transient event and persists through, at least, 7 months of age, which is the oldest age that we have analyzed for population differences. The sustained increased in the number of oligodendrocytes raises the question-- how is this enhanced number of oligodendrocytes achieved and maintained?

Previous studies have demonstrated that sulfatide is a negative regulator of oligodendrocyte development (Bansal et al., 1999; Hirahara et al., 2004). These authors demonstrated that the absence of sulfatide results in an enhanced rate of cellular maturation facilitating the increase in the number of adult oligodendrocytes observed in immature galactolipid null animals. Here, we investigate alternative, although potentially complimentary, mechanisms that may result in the initial and permanent enhancement of the oligodendrocyte population.

One method by which an oligodendrocyte population is expanded is through proliferation (Kierstead and Blakemore, 1999; Miller, 2002; Rowitch, 2004; Menn et al., 2006; Vana et al., 2007). Consistent with this approach for populating the CNS, our findings reveal a significant increase in proliferative activity in the absence of sulfatide. Although we were unable to provide direct evidence that the mitotic cells were of an oligodendrocytic origin, a significant increase in the OPC population is consistent with enhanced proliferation of

oligodendrocyte lineage cells. These findings suggest that sulfatide plays a negative regulatory role in oligodendrocytic proliferation. Consistent with this possibility, the onset of galactolipid synthesis temporally corresponds to the commencement of terminal differentiation which is characterized by the loss of mitotic activity (Hardy and Reynolds, 1991; Pfeiffer et al., 1993). Interestingly, the absence of sulfatide does not abrogate the proliferative activity of oligodendrocyte lineage cells *in vitro* (Bansal et al., 1999). Determining why cells *in vitro* and *in vivo* respond differently to the absence of sulfatide could provide important clues to our understanding of the mechanisms that control oligodendrocyte proliferation.

Since an excess of oligodendrocytes normally enter white matter regions of the CNS only to die as a consequence of insufficient trophic support (Barres et al., 1992; 1993a and b; Trapp et al., 1997), increased proliferation without a mechanisms to support these excess cells would not, intuitively, result in greater cell numbers in adult animals. Thus, we predicted that increased proliferation without corresponding increase in trophic factor availability would merely result in increased cell pruning through apoptosis and any increase in oligodendrocyte population observed in immature animals would be transient. In contrast, if more cells are capable of acquiring the necessary trophic factors, then an increased oligodendrocyte population could persist. Since a current hypothesis is that trophic oligodendrocyte support is obtained through axonal contact (Barres et al., 1992; 1993a and b; Trapp et al., 1997), we proposed that more oligodendrocyte lineage cells are able to establish and maintain axonal contact in the mutant mice. Our hypothesis is strongly supported by our ultrastructural analysis that the sulfatide null oligodendrocytes extend fewer processes per cell. With each cell extending fewer processes, more cells make sufficient axonal contact and avoid apoptosis. Since our previous work demonstrated neither an increase in the number of unmyelinated axons nor a loss of axons until 7 months of age (Marcus et al., 2006) and the number of nodal sodium channel clusters is the same in 4 week old WT and sulfatide null mice (Ishibashi et al., 2002), we conclude that the sulfatide null and WT mice appear to form the same number of myelin segments. With equal demand for myelin formation combined with retarded capacity for process extension by the sulfatide null oligodendrocytes, it appears more mutant cells are required to create an equivalent number of myelin segments and adequately myelinate the CNS.

Does sulfatide regulate oligodendrocyte morphology?

Although the sulfatide null oligodendrocytes form fewer processes *in vivo*, the pathologic mechanism responsible for this morphologic change is not known. The simplest explanation is that these lipid-deficient-oligodendrocytes are limited in their ability to produce membrane. Consistent with this possibility, we have shown that the sulfatide null mice synthesize significantly less (thinner) myelin (Marcus et al., 2006); however, sulfatide only constitutes between 4%-7% of the dry weight of myelin lipids (Norton and Cammer, 1984). Thus, it is unlikely that the deficit in membrane formation results simply from a bulk loss of sulfatide. Instead, it is more probable that a sulfatide mediated signaling event is compromised. This possibility has been previously presented by Bansal et al. (1999) who proposed that sulfatide facilitates a transmembrane signaling cascade required for proper regulation of oligodendrocyte development. Sulfatide concentrates in the outer leaflet of the

oligodendrocyte membrane (Cestaro et al., 1984) and is well positioned to function as a ligand to “transmit environmental information” (Bansal et al., 1988; Bansal and Pfeiffer, 1989). Although the precise cellular response that is induced by galactolipid activation is not known, Benjamins and colleagues (Dyer and Benjamins, 1990; 1991; Benjamins and Dyer, 1990) have provided evidence that these lipids play a role in regulating microtubule organization through a second messenger mediated mechanism. In the absence of sulfatide, microtubule organization may be compromised which could account for not only the reduced number of oligodendrocyte processes but the altered morphology of the processes that are formed (i.e. thick processes that retain cytoplasm, see Figure 6).

In summary, our data demonstrate that the increased number of oligodendrocytes reported by Hirahara et al. (2004) is not a transient event as the enhanced oligodendrocyte population is maintained in the adult sulfatide null animals. Furthermore, we propose that increased proliferation contributes to the production of “extra” oligodendrocytes and a greater proportion of oligodendrocyte lineage cells avoid developmental pruning as a consequence of less complex cellular morphology. It will be of great interest to determine whether a transient reduction of the galactolipids, in particularly sulfatide, can facilitate an enhanced response to demyelinating events.

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References

Bansal R, Gard AL, Pfeiffer SE. Stimulation of oligodendrocyte differentiation in culture by growth in the presence of a monoclonal antibody to sulfated glycolipid. J Neurosci Res. 1988; 21(2-4):260–7. [PubMed: 2464078]

Bansal R, Pfeiffer SE. Reversible inhibition of oligodendrocyte progenitor differentiation by a monoclonal antibody against surface galactolipids. Proc Natl Acad Sci U S A. 1989; 86(16):6181–5. [PubMed: 2668957]

Bansal R, Warrington AE, Gard AL, Ranscht B, Pfeiffer SE. Multiple and novel specificities of monoclonal antibodies O1, O4, and R-mAb used in the analysis of oligodendrocyte development. J Neurosci Res. 1989; 24(4):548–57. [PubMed: 2600978]

Bansal R, Pfeiffer SE. Inhibition of protein and lipid sulfation in oligodendrocytes blocks biological responses to FGF-2 and retards cytoarchitectural maturation, but not developmental lineage progression. Dev Biol. 1994; 162(2):511–24. [PubMed: 8150210]

Bansal R, Winkler S, Bheddah S. Negative regulation of oligodendrocyte differentiation by galactosphingolipids. J Neurosci. 1999; 19(18):7913–24. [PubMed: 10479693]

Barres BA, Hart IK, Coles HS, Burne JF, Voyvodic JT, Richardson WD, Raff MC. Cell death and control of cell survival in the oligodendrocyte lineage. Cell. 1992; 70(1):31–46. [PubMed: 1623522]

Barres BA, Jacobson MD, Schmid R, Sendtner M, Raff MC. Does oligodendrocyte survival depend on axons? Curr Biol. 1993a; 3(8):489–97. [PubMed: 15335686]

Barres BA, Schmid R, Sendnter M, Raff MC. Multiple extracellular signals are required for long-term oligodendrocyte survival. Development. 1993b; 118(1):283–95. [PubMed: 8375338]

Benjamins JA, Dyer CA. Glycolipids and transmembrane signaling in oligodendroglia. Ann N Y Acad Sci. 1990; 605:90–100. [PubMed: 2268123]

Bhat RV, Axt KJ, Fosnaugh JS, Smith KJ, Johnson KA, Hill DE, Kinzler KW, Baraban JM. Expression of the APC tumor suppressor protein in oligodendroglia. Glia. 1996; 17(2):169–74. [PubMed: 8776583]

Boggs JM, Wang H. Effect of liposomes containing cerebroside and cerebroside sulfate on cytoskeleton of cultured oligodendrocytes. J Neurosci Res. 2001; 66(2):242–53. [PubMed: 11592120]

Bosio A, Binczek E, Stoffel W. Functional breakdown of the lipid bilayer of the myelin membrane in central and peripheral nervous system by disrupted galactocerebroside synthesis. Proc Natl Acad Sci U S A. 1996; 93(23):13280–5. [PubMed: 8917582]

Cestaro B, Marchesini S, Cervato G, Viani P, Vesely S. Bilayer-micelle transition in phosphatidylcholine-sulfatide mixtures. Ital J Biochem. 1984; 33(6):381–91. [PubMed: 6526641]

Coetzee T, Fujita N, Dupree J, Shi R, Blight A, Suzuki K, Suzuki K, Popko B. Myelination in the absence of galactocerebroside and sulfatide: normal structure with abnormal function and regional instability. Cell. 1996; 86(2):209–19. [PubMed: 8706126]

Dubois-Dalcq M, Niedieck B, Buyse M. Action of anti-cerebroside sera on myelinated nervous tissue cultures. Pathol Eur. 1970; 5(3):331–47. [PubMed: 5477028]

Dupree JL, Coetzee T, Blight A, Suzuki K, Popko B. Myelin galactolipids are essential for proper node of Ranvier formation in the CNSn. J Neurosci. 1998; 18(5):1642–9. [PubMed: 9464989]

Dupree JL, Popko B. Genetic dissection of myelin galactolipid function. J Neurocytol. 1999; 28(4-5): 271–9. [PubMed: 10739570]

Dupree JL, Mason JL, Marcus JR, Stull M, Levinson R, Matsushima GK, Popko B. Oligodendrocytes assist in the maintenance of sodium channel clusters independent of the myelin sheath. Neuron Glia Biol. 2005; 1:1–14. [PubMed: 16528406]

Dyer CA, Benjamins JA. Glycolipids and transmembrane signaling: antibodies to galactocerebroside cause an influx of calcium in oligodendrocytes. J Cell Biol. 1990; 111(2):625–33. [PubMed: 2166054]

Dyer CA, Benjamins JA. Galactocerebroside and sulfatide independently mediate Ca2+ responses in oligodendrocytes. J Neurosci Res. 1991; 30(4):699–711. [PubMed: 1787543]

Fry JM, Weissbarth S, Lehrer GM, Bornstein MB. Cerebroside antibody inhibits sulfatide synthesis and myelination and demyelinates in cord tissue cultures. Science. 1974; 183(124):540–2. [PubMed: 4203489]

Fuss B, Mallon B, Phan T, Ohlemeyer C, Kirchhoff F, Nishiyama A, Macklin WB. Purification and analysis of in vivo-differentiated oligodendrocytes expressing the green fluorescent protein. Dev Biol. 2000; 218(2):259–74. [PubMed: 10656768]

Gokhan S, Marin-Husstege M, Yung SY, Fontanez D, Casaccia-Bonnefil P, Mehler MF. Combinatorial profiles of oligodendrocyte-selective classes of transcriptional regulators differentially modulate myelin basic protein gene expression. J Neurosci. 2005; 25(36):8311–21. [PubMed: 16148239]

Hardy R, Reynolds R. Proliferation and differentiation potential of rat forebrain oligodendroglial progenitors both in vitro and in vivo. Development. 1991; 111(4):1061–80. [PubMed: 1879350]

Hirahara Y, Bansal R, Honke K, Ikenaka K, Wada Y. Sulfatide is a negative regulator of oligodendrocyte differentiation: development in sulfatide-null mice. Glia. 2004; 45(3):269–77. [PubMed: 14730700]

Honke K, Hirahara Y, Dupree J, Suzuki K, Popko B, Fukushima K, Fukushima J, Nagasawa T, Yoshida N, Wada Y, Taniguchi N. Paranodal junction formation and spermatogenesis require sulfoglycolipids. Proc Natl Acad Sci U S A. 2002; 99(7):4227–32. [PubMed: 11917099]

Ishibashi T, Dupree JL, Ikenaka K, Hirahara Y, Honke K, Peles E, Popko B, Suzuki K, Nishino H, Baba H. A myelin galactolipid, sulfatide, is essential for maintenance of ion channels on myelinated axon but not essential for initial cluster formation. J Neurosci. 2002; 22(15):6507–14. [PubMed: 12151530]

Keirstead HS, Blakemore WF. The role of oligodendrocytes and oligodendrocyte progenitors in CNS remyelination. Adv Exp Med Biol. 1999; 468:183–97. [PubMed: 10635029]

Ligon KL, Alberta JA, Kho AT, Weiss J, Kwaan MR, Nutt CL, Louis DN, Stiles CD, Rowitch DH. The oligodendroglial lineage marker OLIG2 is universally expressed in diffuse gliomas. J Neuropathol Exp Neurol. 2004; 63(5):499–509. [PubMed: 15198128]

Lu QR, Yuk D, Alberta JA, Zhu Z, Pawlitzky I, Chan J, McMahon AP, Stiles CD, Rowitch DH. Sonic hedgehog--regulated oligodendrocyte lineage genes encoding bHLH proteins in the mammalian central nervous system. Neuron. 2000 Feb; 25(2):317–29. 2000. [PubMed: 10719888]

Marcus J, Honigbaum S, Shroff S, Honke K, Rosenbluth J, Dupree JL. Sulfatide is essential for the maintenance of CNS myelin and axon structure. Glia. 2006; 53(4):372–81. [PubMed: 16288467]

Marcus J, Dupree JL, Popko B. Effects of galactolipid elimination on oligodendrocyte development and myelination. Glia. 2000; 30(4):319–28. [PubMed: 10797612]

Masahira N, Takebayashi H, Ono K, Watanabe K, Ding L, Furusho M, Ogawa Y, Nabeshima Y, Alvarez-Buylla A, Shimizu K, Ikenaka K. Olig2-positive progenitors in the embryonic spinal cord give rise not only to motoneurons and oligodendrocytes, but also to a subset of astrocytes and ependymal cells. Dev Biol. 2006; 293(2):358–69. [PubMed: 16581057]

Messersmith DJ, Murtie JC, Le TQ, Frost EE, Armstrong RC. Fibroblast growth factor 2 (FGF2) and FGF receptor expression in an experimental demyelinating disease with extensive remyelination. J Neurosci Res. 2000; 62(2):241–56. [PubMed: 11020217]

Menn B, Garcia-Verdugo JM, Yaschine C, Gonzalez-Perez O, Rowitch D, Alvarez-Buylla A. Origin of oligodendrocytes in the subventricular zone of the adult brain. J Neurosci. 2006; 26(30):7907–18. [PubMed: 16870736]

Miller RH. Regulation of oligodendrocyte development in the vertebrate. CNS Prog Neurobiol. 2002; 67(6):451–67. [PubMed: 12385864]

Molander-Melin M, Pernber Z, Franken S, Gieselmann V, Månsson JE, Fredman P. Accumulation of sulfatide in neuronal and glial cells of arylsulfatase A deficient mice. J Neurocytol. 2004; 33(4): 417–27. [PubMed: 15520527]

Murtie JC, Zhou YX, Le TQ, Armstrong RC. In vivo analysis of oligodendrocyte lineage development in postnatal FGF2 null mice. Glia. 2005; 49(4):542–54. [PubMed: 15578654]

Nait-Oumesmar B, Picard-Riera N, Kerninon C, Decker L, Seilhean D, Höglinger GU, Hirsch EC, Reynolds R, Baron-Van Evercooren A. Activation of the subventricular zone in multiple sclerosis: evidence for early glial progenitors. Proc Natl Acad Sci USA. 2007; 104(11):4694–9. [PubMed: 17360586]

Norton, W.; Cammer, W. Isolation and characterization of myelin. In: Morell, P., editor. Myelin. New York: Plenum; 1984. p. 147-180.

Owens GC, Bunge RP. Schwann cells depleted of galactocerebroside express myelin-associated glycoprotein and initiate but do not continue the process of myelination. Glia. 1990; 3(2):118–24. [PubMed: 1692007]

Pernber Z, Molander-Melin M, Berthold CH, Hansson E, Fredman P. Expression of the myelin and oligodendrocyte progenitor marker sulfatide in neurons and astrocytes of adult rat brain. J Neurosci Res. 2002; 69(1):86–93. [PubMed: 12111819]

Pernet V, Joly S, Christ F, Dimou L, Schwab ME. Nogo-a and myelin-associated glycoprotein differently regulate oligodendrocyte maturation and myelin formation. J Neurosci. 2008; 28(29): 7435–44. [PubMed: 18632947]

Pfeiffer SE, Warrington AE, Bansal R. The oligodendrocyte and its many cellular processes. Trends Cell Biol. 1993; 3(6):191–7. [PubMed: 14731493]

Ranscht B, Clapshaw PA, Price J, Noble M, Seifert W. Development of oligodendrocytes and Schwann cells studied with a monoclonal antibody against galactocerebroside. Proc Natl Acad Sci USA. 1982; 79(8):2709–13. [PubMed: 7045870]

Ranscht B, Wood PM, Bunge RP. Inhibition of in vitro peripheral myelin formation by monoclonal anti-galactocerebroside. J Neurosci. 1987; 7(9):2936–47. [PubMed: 3625279]

Rosenbluth J, Liu Z, Guo D, Schiff R. Inhibition of CNS myelin development in vivo by implantation of anti-GalC hybridoma cells. J Neurocytol. 1994; 23(11):699–707. [PubMed: 7861184]

Rosenbluth J, Liang WL, Liu Z, Guo D, Schiff R. Paranodal structural abnormalities in rat CNS myelin developing in vivo in the presence of implanted O1 hybridoma cells. Brain Res. 1995; 686(2):143–9. [PubMed: 7583280]

Roth GA, Röyttä M, Yu RK, Raine CS, Bornstein MB. Antisera to different glycolipids induce myelin alterations in mouse spinal cord tissue cultures. Brain Res. 1985; 339(1):9–18. [PubMed: 4027618]

Rowitch DH. Glial specification in the vertebrate neural tube. Nat Rev Neurosci. 2004; 5(5):409–19. [PubMed: 15100723]

Saida K, Saida T, Brown MJ, Silberberg DH. In vivo demyelination induced by intraneural injection of anti-galactocerebroside serum: a morphologic study. Am J Pathol. 1979; 95(1):99–116. [PubMed: 434114]

Saito M, Macala LJ, Roth GA, Bornstein MB, Yu RK. Effect of antiglycolipid antisera on the lipid composition of cultured mouse spinal cords. Exp Neurol. 1986; 92(3):752–6. [PubMed: 3709746]

Sergott RC, Brown MJ, Silberberg DH. Remyelination follows antibody-induced central nervous system demyelination. Ann Neurol. 1986; 20(1):94–8. [PubMed: 3740817]

Sohn J, Natale J, Chew LJ, Belachew S, Cheng Y, Aguirre A, Lytle J, Nait-Oumesmar B, Kerninon C, Kanai-Azuma M, Kanai Y, Gallo V. Identification of Sox17 as a transcription factor that regulates oligodendrocyte development. J Neurosci. 2006; 26(38):9722–35. [PubMed: 16988043]

Taylor, CM.; Marta, CB.; Bansal, R.; Pfeiffer, SE. The Transport, Assembly, and Function of Myelin Lipids. In: Lazzarini, RA., editor. Myelin Biology and Disorders. Vol. 1. Academic Press; 2004. p. 57-88.

Trapp BD, Nishiyama A, Cheng D, Macklin W. Differentiation and death of premyelinating oligodendrocytes in developing rodent brain. J Cell Biol. 1997; 137(2):459–68. [PubMed: 9128255]

Vana AC, Lucchinetti CF, Le TQ, Armstrong RC. Myelin transcription factor 1 (Myt1) expression in demyelinated lesions of rodent and human. CNS Glia. 2007; 55(7):687–97. [PubMed: 17330875]

Zhou Q, Wang S, Anderson DJ. Identification of a novel family of oligodendrocyte lineage-specific basic helix-loop-helix transcription factors. Neuron. 2000; 25(2):331–43. [PubMed: 10719889]

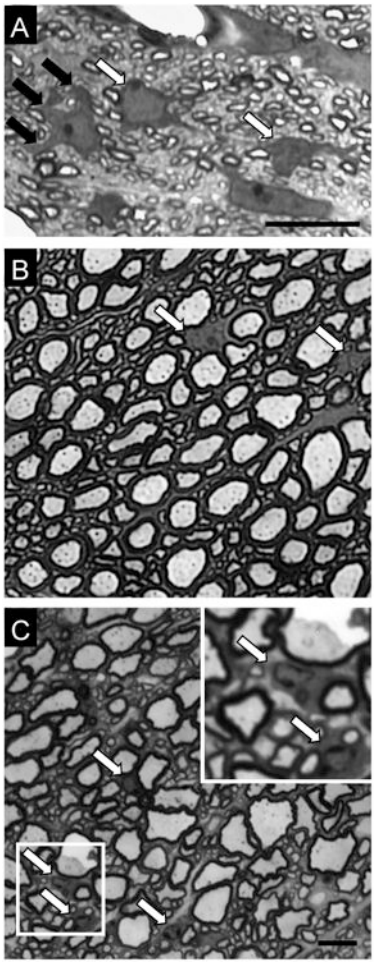

Figure 1.

A) Immature oligodendrocytes (white, block arrows) have prominent nuclei, a thin rim of cytoplasm and extend multiple myelin forming, primary (black, block arrow) processes. Note that the primary processes have the potential to divide and form secondary processes (black, line arrows) that are capable of myelinating axons. B) In the ventral columns of the WT adult spinal cord, myelinated axons are the most prominent structure. Occasionally, nuclei (black with white outlined, block arrows) are observed among the numerous myelinated axons. In contrast to the adult WT spinal cord, littermate sulfatide null mice **(C)** display numerous nuclei (black with white outline, block arrows) positioned among the myelinated fibers. The inset in Panel C displays 2 cytoplasm-containing cells that maintain intimate association with small, myelinated axons. These cells reveal morphologies consistent with myelinating oligodendrocytes. Scale bars = 10μm in Panel A and 20 μm is Panels B and C.

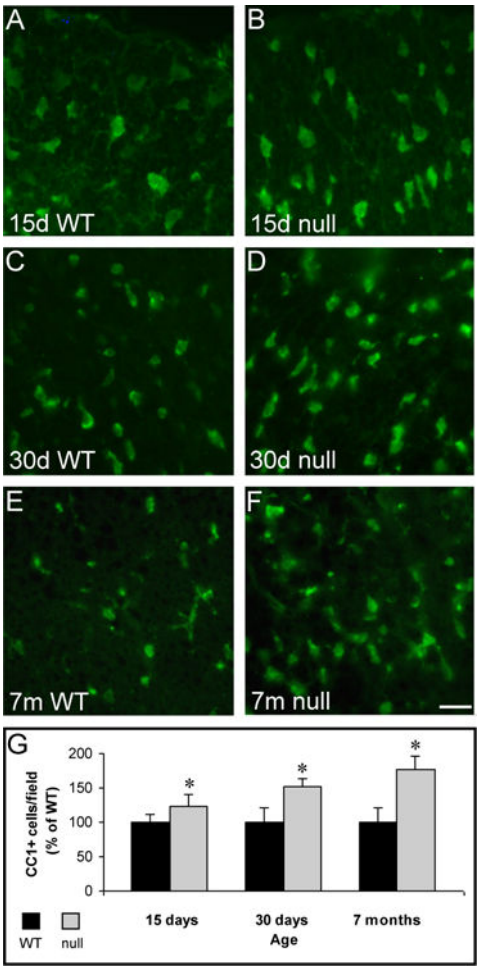

Figure 2.

Immunolabeling with the mature oligodendrocyte marker CC1 revealed that the ventral column of the spinal cord from the WT mice (A, C, and E) maintained fewer oligodendrocytes than littermate sulfatide null mice (B, D, and F) at 15 days, 30 days and 7 months of age. Based on statistical analyses with a 2-tailed t test, the number of oligodendrocytes in the spinal cord was significantly greater in the sulfatide null mice than the WT mice at all ages studied (G). Bars on graph represent mean number of cells per microscopic field ± standard deviation. Scale bar = 20 μm

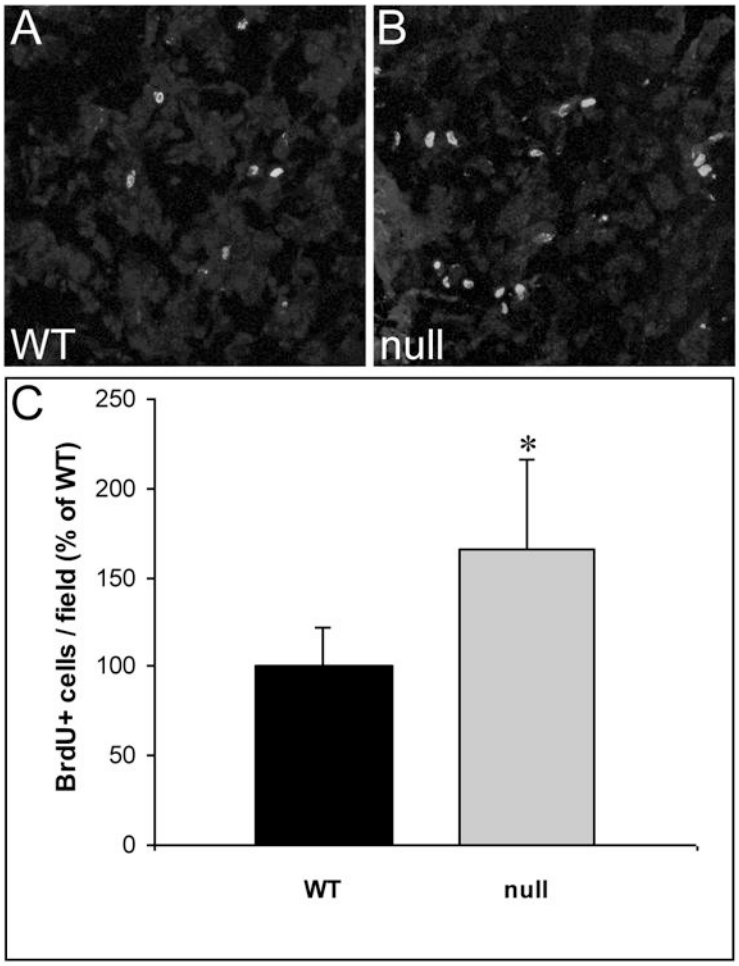

Figure 3.

BrdU+ cells were observed in the ventral column white matter tracts of both the WT (A) and littermate sulfatide null (B) mice. Quantitative analysis revealed significantly fewer BrdU+ cells in the spinal cord ventral columns of the sulfatide null mice compared to littermate WT animals. Values represent mean number of cells per microscopic field expressed as a percent of WT ± percent standard deviation. Scale bar = 100 μm.

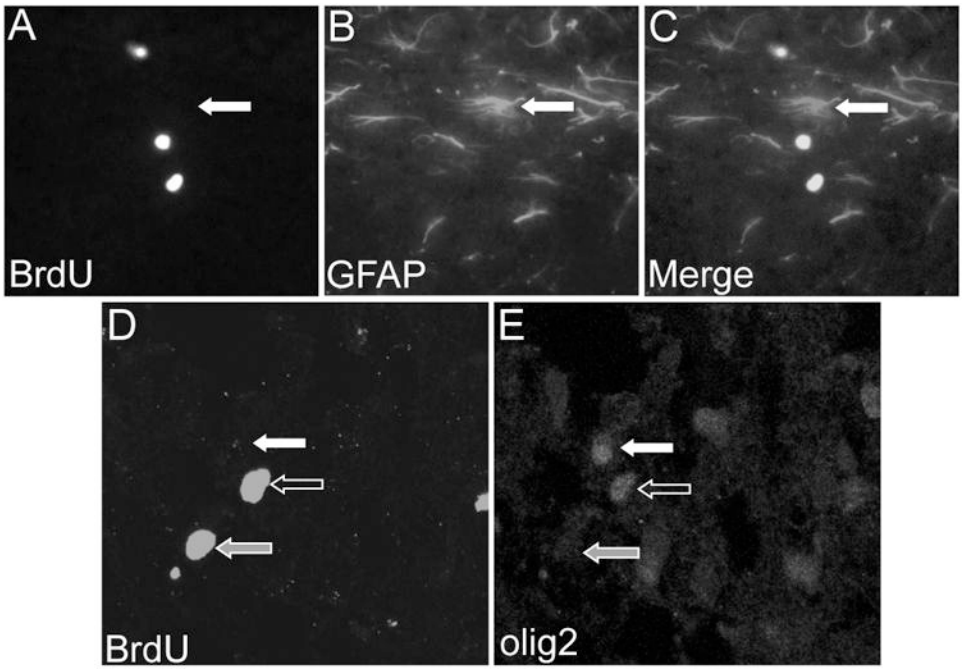

Figure 4.

Panels A, B and C present the same microscopic field with each panel displaying a single label of DAPI (A), olig2 (B) or CC1 (C). Cells that are DAPI+, olig2+/CC1+ (white arrowheads in A, B, and C) were identified as mature oligodendrocytes whereas cells that were DAPI+/olig2+/CC1- were identified as oligodendrocyte progenitor cells (white arrows in A, B and C). D) Significantly more progenitor cells were observed in the ventral columns of the spinal cord in the sulfatide null mice than in the same region of the WT littermates ($p=0.038$). Scale bar = 20 μm.

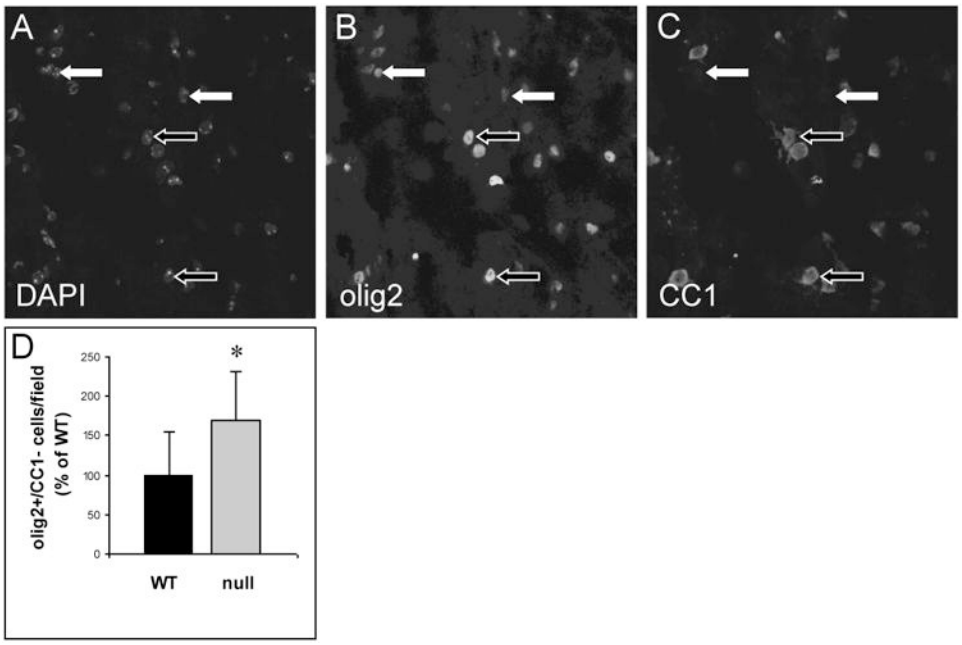

Figure 5.

Panels A, B and C present the same microscopic field with each panel displaying single labeling of DAPI (A), olig2 (B) or TUNEL reagent (C). These three panels display the feasibility of this approach to distinguish between olig2+ healthy cells (white star in Panels A, B, and C) and olig2+ cells that are undergoing apoptosis (white line arrows in Panels A, B, and C). Also note that olig2- healthy cells were also observed (white block arrow in Panels A, B, and C). Quantitative analysis revealed that significantly fewer DAPI+/olig2+/TUNEL+ cells were observed in the sulfatide null spinal cord ventral columns than in the spinal cord ventral columns of littermate WT mice (D; p=0.02). Values are presented as mean number of cells per microscopic field as a percent of WT ± percent standard deviation. Scale bar = 10 μm.

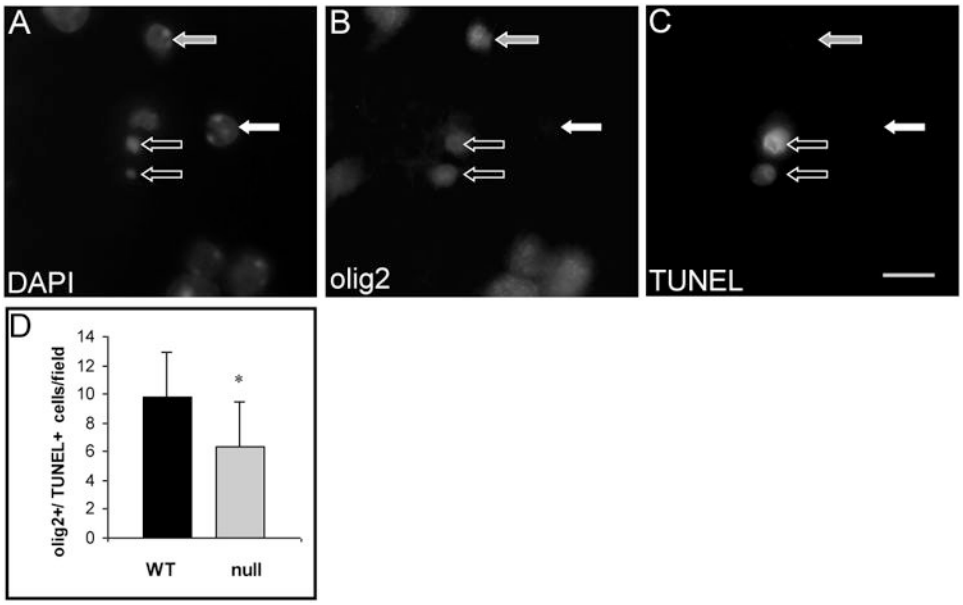

Figure 6.

Oligodendrocytes from the sulfatide null mice (B) extend significantly fewer myelin-forming primary processes than oligodendrocytes from littermate WT animals (A; $p \leq 0.05$). C) Plotting process number against cell frequency for each genotype reveals a dramatic leftward shift for the sulfatide null oligodendrocytes at PND 2. Note that the graph reveals that the mode of process number for the sulfatide null oligodendrocytes is 1 which is substantially less than the mode for the WT oligodendrocytes which is 4. Scale bar = 2 μm.

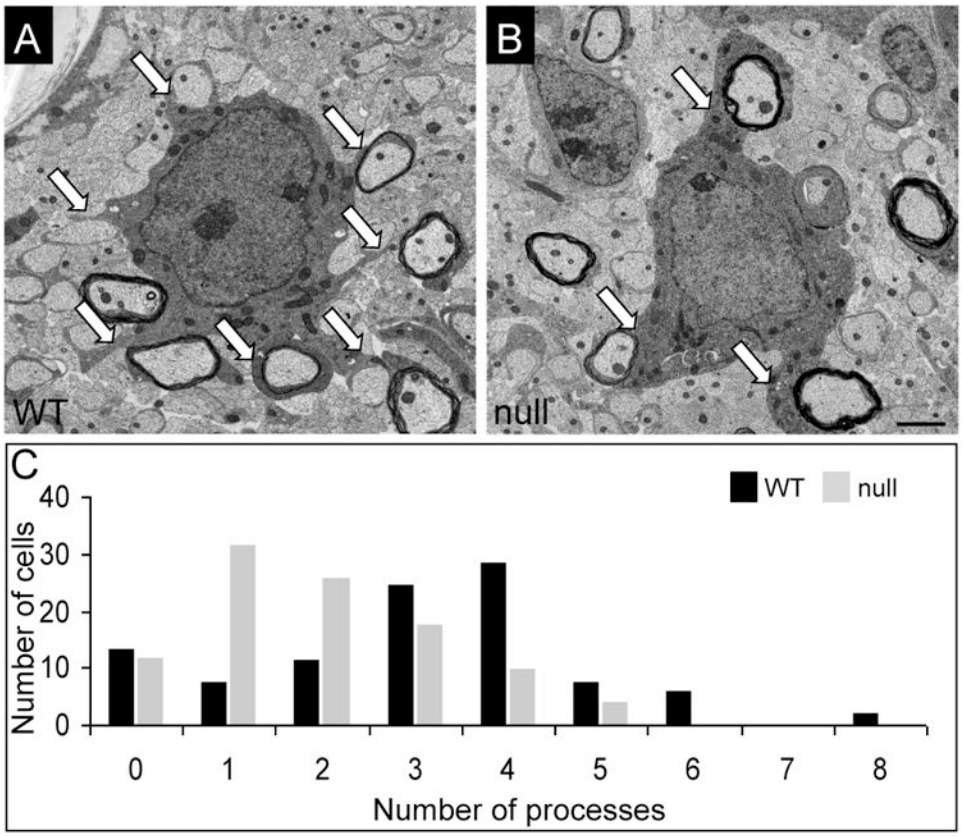

Figure 7. CST-null OLs extend fewer myelin-forming processes than their WT counterparts

Ultrastructural analysis of WT and CST-null OLs revealed that the CNS myelin forming cells from the CST-null mice (B) extend significantly fewer primary processes than OLs from littermate WT animals (A; $p \leq 0.05$). C) Plotting process number against cell frequency for each genotype reveals a dramatic leftward shift for the CST null OLs at PND 2. Note that the graph reveals that the mode of process number for the CST-null OLs is 1 which is substantially less than the mode for the WT OLs which is 4. n=91 cells collected from 3 WT mice and 89 cells collected from 2 CST-null mice; Scale bar = 2 μm.