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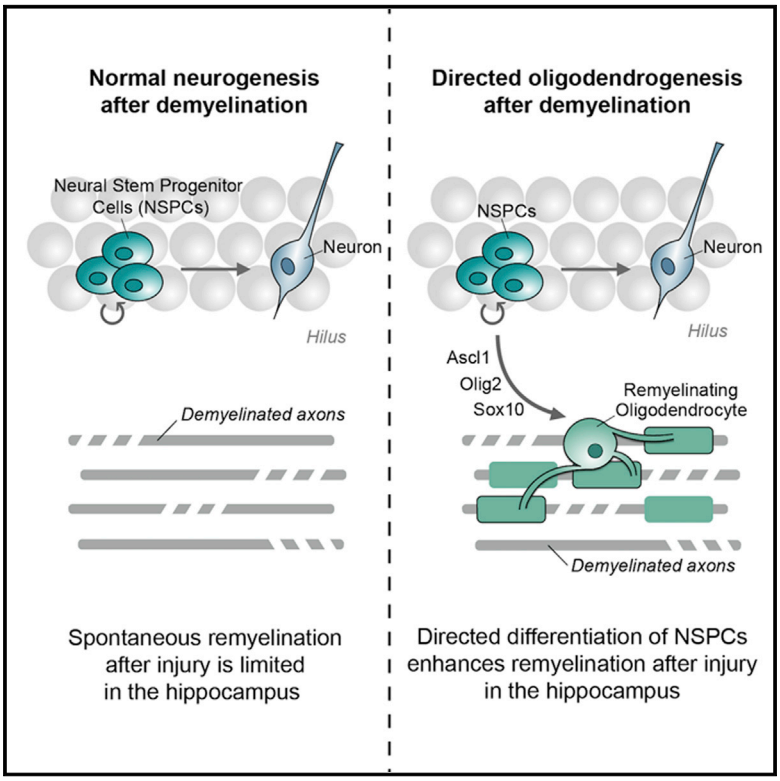
Report

Programming Hippocampal Neural Stem/Progenitor Cells into Oligodendrocytes Enhances Remyelination in the Adult Brain after Injury

Graphical Abstract



Authors

Simon M.G. Braun, Gregor-Alexander Pilz, Raquel A.C. Machado, ..., Burkhard Becher, Nicolas Toni, Sebastian Jessberger

Correspondence

jessberger@hifo.uzh.ch

In Brief

Regenerative approaches for replacing lost oligodendrocytes in demyelinating disease are scant. Braun et al. show that programming adult hippocampal neural stem/progenitor cells (NSPCs) into oligodendrocytes enhances remyelination in a genetic model of demyelination, highlighting the potential of targeting hippocampal NSPCs for the treatment of demyelinated lesions.

Highlights

- Programming hippocampal NSPCs into oligodendrocytes follows developmental programs
- Programming NSPCs into oligodendrocytes enhances remyelination after injury
- Induced oligodendrocytes mature and myelinate, as shown at a single-cell level
- Proof of concept for targeting hippocampal NSPCs for glial brain repair is provided

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Programming Hippocampal Neural Stem/Progenitor Cells into Oligodendrocytes Enhances Remyelination in the Adult Brain after Injury

Simon M.G. Braun,[1,2,5] Gregor-Alexander Pilz,[1,2] Raquel A.C. Machado,[1] Jonathan Moss,[3] Burkhard Becher,[2,4] Nicolas Toni,[3] and Sebastian Jessberger[1,2,*]
[1]Brain Research Institute, Faculty of Medicine and Science, University of Zurich, 8057 Zurich, Switzerland
[2]Neuroscience Center Zurich, University of Zurich and ETH, 8057 Zurich, Switzerland
[3]Department of Fundamental Neurosciences, University of Lausanne, 1015 Lausanne, Switzerland
[4]Institute of Experimental Immunology, University of Zurich, 8057 Zurich, Switzerland
[5]Present address: Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305, USA
*Correspondence: jessberger@hifo.uzh.ch
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SUMMARY

Demyelinating diseases are characterized by a loss of oligodendrocytes leading to axonal degeneration and impaired brain function. Current strategies used for the treatment of demyelinating disease such as multiple sclerosis largely rely on modulation of the immune system. Only limited treatment options are available for treating the later stages of the disease, and these treatments require regenerative therapies to ameliorate the consequences of oligodendrocyte loss and axonal impairment. Directed differentiation of adult hippocampal neural stem/progenitor cells (NSPCs) into oligodendrocytes may represent an endogenous source of glial cells for cell-replacement strategies aiming to treat demyelinating disease. Here, we show that Ascl1-mediated conversion of hippocampal NSPCs into mature oligodendrocytes enhances remyelination in a diphtheria-toxin (DT)-inducible, genetic model for demyelination. These findings highlight the potential of targeting hippocampal NSPCs for the treatment of demyelinated lesions in the adult brain.

INTRODUCTION

Neural stem/progenitor cells (NSPCs) reside in two distinct regions of the adult mammalian brain: the subventricular zone (SVZ) and the hippocampal dentate gyrus (DG) (Gage, 2000). Harnessing the regenerative capacity of endogenous adult NSPCs can contribute to brain repair (Lindvall and Kokaia, 2006). Previous reports have suggested the remyelination potential of NSPC-derived oligodendrocytes in areas neighboring the adult SVZ (Menn et al., 2006; Nakatani et al., 2013; Rafalski et al., 2013). However, in striking contrast to the SVZ, NSPCs in the DG do not spontaneously differentiate into oligodendrocytes and remyelination is limited after injury in the hippocampal formation. The hippocampus plays a pivotal role in certain forms of learning and memory and is commonly affected in demyelinating diseases such as multiple sclerosis as well as other brain disorders, including traumatic brain injury, epilepsy, Alzheimer's disease, and schizophrenia (Chambers and Perrone-Bizzozero, 2004; Hemanth Kumar et al., 2014; Meier et al., 2004; Noble, 2004; Yang et al., 2009; Chiaravalloti and DeLuca, 2008; Geurts et al., 2007), underlining the need for therapies that promote remyelination within this brain region (Franklin and Ffrench-Constant, 2008). Overexpression of the transcription factor (TF) Ascl1 converts hippocampal NSPCs into oligodendrocytic cells in vivo (Jessberger et al., 2008), but their potential for remyelination remains unknown (Goldman and Natesan, 2008; Jessberger and Gage, 2009). Thus, we here evaluate the remyelination capacity of hippocampal NSPCs by in vivo reprogramming of hippocampal NSPCs into oligodendrocytes in a mouse model of demyelinating disease.

RESULTS

Overexpression of Ascl1, Olig2, or Sox10 TFs Directs Hippocampal NSPC Differentiation toward the Oligodendrocyte Lineage In Vivo

To identify novel TFs that direct NSPC progeny toward the oligodendrocyte lineage, we focused on a developmental pathway that plays an important role in oligodendrogenesis during brain development (Nakatani et al., 2013). Ascl1 is required for oligodendrogenesis through a genetic interaction with Olig2, an essential transcriptional regulator of oligodendrocyte fate (Petryniak et al., 2007; Zhou and Anderson, 2002). Furthermore, Olig2 binds regulatory elements to induce the expression of Sox10, a TF that is required for expression of myelin genes (Küspert et al., 2011; Stolt et al., 2002). First, we transduced adult hippocampal NSPCs in vitro using retroviruses overexpressing Ascl1, Olig2, or Sox10 and analyzed cell fates under differentiating conditions. We found that overexpression of either of these TFs increased oligodendrocyte differentiation of adult NSPCs when compared to cells infected with control retroviruses (Figures

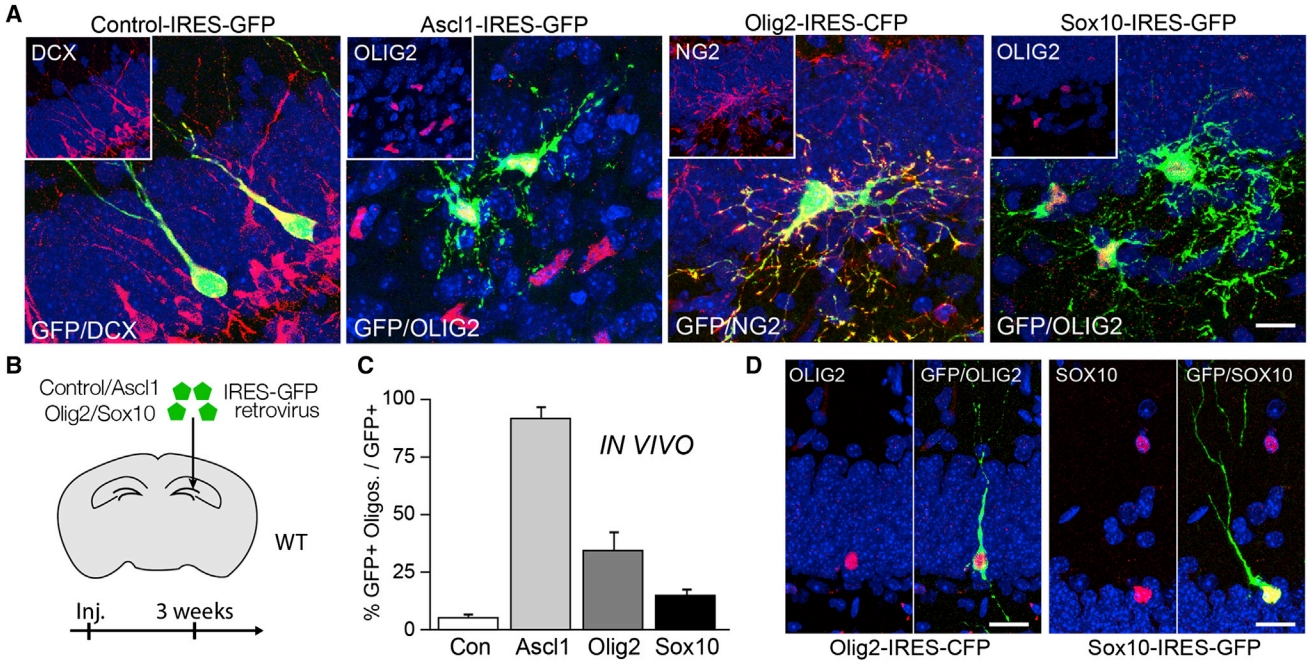

Figure 1. Retroviral Overexpression of Ascl1, Olig2, and Sox10 in Adult Hippocampal NSPCs In Vivo

(A) Wild-type adult mice were injected with control, Ascl1-, Olig2-, or Sox10-overexpressing retroviruses into the DG and analyzed 3 weeks later. The fate of labeled NSPCs was determined by staining for oligodendroglial (OLIG2 or NG2, red) and neuronal (DCX, red) markers (n = 3 for each TF).

(B) Scheme describing the experimental setup.

(C) Graph showing the percentage of GFP-expressing cells positive for the oligodendrocyte markers OLIG2 or NG2 for each condition (>100 cells were phenotyped per condition).

(D) Overexpression of Olig2 or Sox10 in hippocampal NSPCs increases oligodendrocyte differentiation by 6.8-fold and 2.9-fold, respectively. However, many NSPCs differentiate into neurons despite Olig2 and Sox10 overexpression, although neuronal differentiation appears to be abnormal as shown by the aberrant morphology of the dendritic processes.

Error bars represent mean ± SEM. Scale bars represent 40 μm. Nuclei were stained with DAPI (blue).

S1A–S1C). Sox10 was the most potent inducer of oligodendrocyte differentiation, whereas Ascl1 and Olig2 overexpression only modestly increased oligodendrogenesis in vitro (Figure S1D). Given the importance of niche-derived factors for directed differentiation of hippocampal NSPCs (Goldman and Natesan, 2008; Jessberger and Gage, 2009), we injected Ascl1-, Olig2-, or Sox10-expressing retroviruses directly into the DG of adult mice to determine their reprogramming efficiencies in vivo. Interestingly, Olig2 and Sox10 TFs were able to significantly enhance NSPC differentiation into oligodendrocytes in vivo (Figures 1A–1D), albeit at lower efficiencies than Ascl1. These findings indicate that the fate potential of hippocampal NSPCs remains highly plastic, as the induction of a developmental genetic program at different stages redirects the fate of adult NSPCs toward the oligodendrocyte lineage.

Spontaneous Remyelination Is Limited in the Hippocampus after DT-Induced Ablation of Mature Oligodendrocytes

Next, we developed an inducible model for focal demyelination to test if directed differentiation of NSPCs into oligodendrocytes, using the most potent TF Ascl1, can enhance remyelination in the hippocampus. To ablate oligodendrocytes in the adult brain, we crossed mice expressing an oligodendrocyte-specific Cre recombinase (MOG-Cre) with mice harboring a Cre-inducible diphtheria toxin receptor (DTR) (Buch et al., 2005). The resulting oDTR mice (MOGCre$^{+/-}$/DTR$^{+/-}$) express the DTR specifically in mature oligodendrocytes, rendering these cells sensitive to DT-induced cell death (Figure 2A). For focal demyelination, we stereotactically injected DT into the DG of adult oDTR mice. We observed a substantial decrease in myelin basic protein (MBP) levels within the hippocampus of oDTR mice when compared to their littermate controls (Con: MOGCre$^{-/-}$/DTR$^{+/-}$) (Figure 2B). These results validate this novel DT-inducible genetic approach for focal ablation of oligodendrocytes and subsequent demyelination in discreet brain areas. Myelin levels remained significantly lower in oDTR mice compared to controls 3, 6, and 10 weeks after injury and did not increase over time, indicating that focal DT injection leads to chronic demyelination in oDTR mice (Figures 2C–2E). In support of this finding, we observed that oligodendrocyte precursor cell (OPC) numbers remained unchanged in the hilus 3 weeks after DT injection (Figures S2A–S2C). In addition, the fate of newborn cells was not altered after demyelination, as in both oDTR and control mice, the vast majority of NSPCs differentiated into neurons (Figures S2D–S2F), suggesting that demyelination of the hippocampus is not sufficient to direct the fate of NSPCs into oligodendrocytes. Taken together these results indicate that endogenous remyelination is limited in

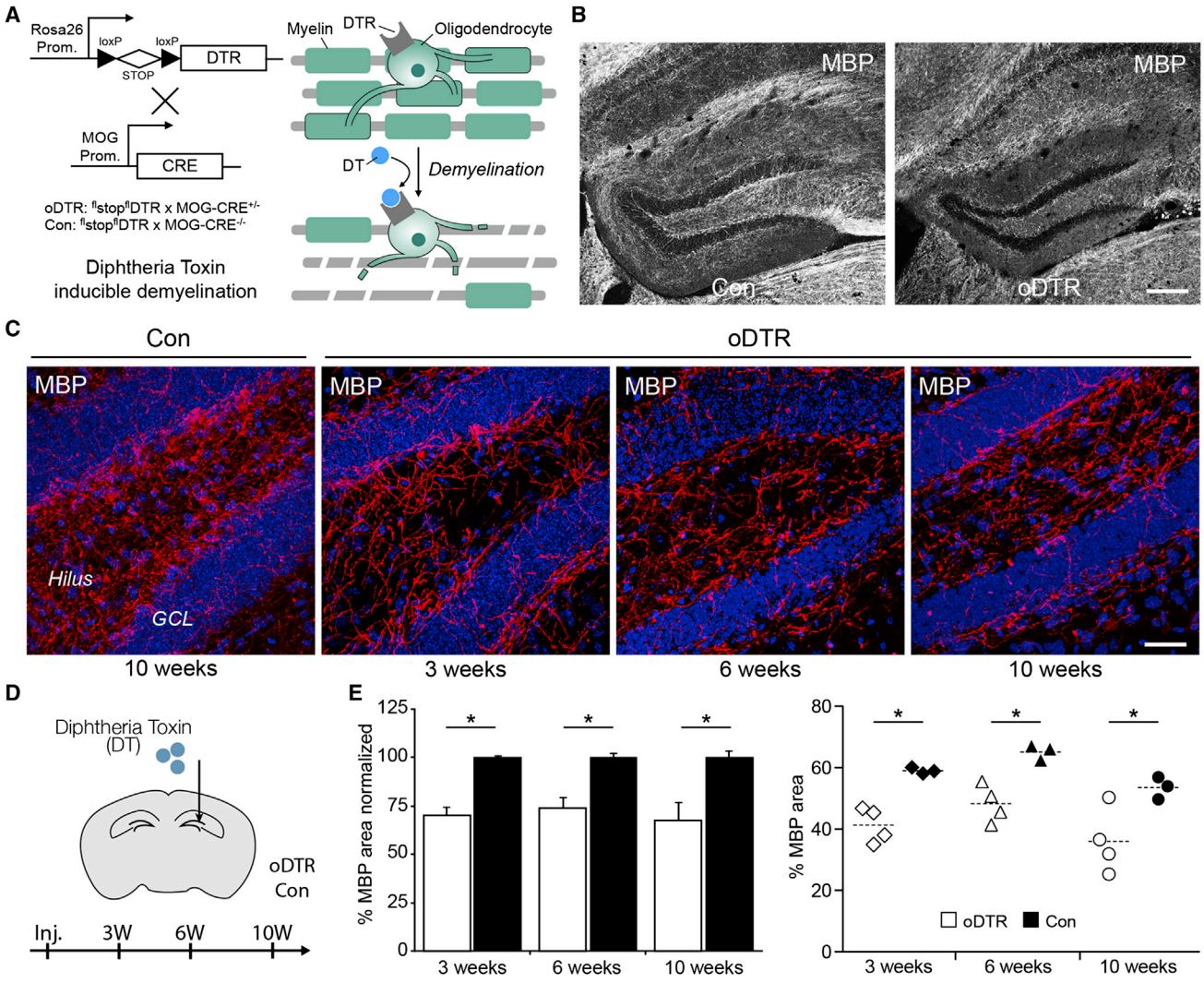

Figure 2. Focal Ablation of Oligodendrocytes in the Hippocampus of Adult oDTR Mice

(A) The oDTR mouse model was generated by crossing DTR mice with MOG-Cre mice to allow for DT-inducible ablation of oligodendrocytes and subsequent demyelination in the adult brain.

(B) Stereotactic delivery of DT leads to focal ablation of oligodendrocytes. Loss of myelin (MBP, white) was restricted to the hippocampus of oDTR mice after injection of 0.5 ng DT in the DG. Control mice lacking MOG-Cre expression do not express the DTR, rendering them insensitive to DT-mediated ablation of oligodendrocytes.

(C) oDTR and Con mice were injected with DT in the DG and MBP levels (red) in the hilus of the DG were determined 3, 6, and 10 weeks after the injections.

(D) Scheme describing the experimental setup.

(E) Graphs showing the quantification of hilar MBP levels in oDTR and Con mice at different time points. The left panel shows %MBP area normalized to control. The right panel shows measured %MBP areas. (oDTR n = 4 and Con n = 3, for each time point).

Error bars represent mean ± SEM. Scale bars represent 10 μm (B) and 40 μm (C). Nuclei were stained with DAPI (blue). GCL, granule cell layer.

the DG of the hippocampus following the ablation of mature oligodendrocytes.

Directed Differentiation of NSPCs into Oligodendrocytes Increases Myelin Levels in the Adult Hippocampus after Injury

To evaluate the remyelination potential of fate-directed hippocampal NSPC progeny, we injected Ascl1-IRES-GFP expressing retroviruses into the DG of demyelinated oDTR mice to convert the differentiation of NSPCs into oligodendrocytes, as this TF showed the highest reprogramming efficiency. We found that in a focally demyelinated hippocampus, Ascl1 overexpression efficiently converted NSPCs into oligodendrocytes (95.25% ± 0.84%) when retroviruses were co-injected into the DG together with DT toxin to induce demyelination. NSPC-derived oligodendrocytes (GFP+/OLIG2+) were observed throughout the demyelinated hilus of the DG but remained within the DG boundaries (Figure 3A). Demyelination of the hippocampus did not alter proliferation levels of converted NSPCs, and proliferation of GFP-negative, endogenous OPCs was not significantly enhanced in

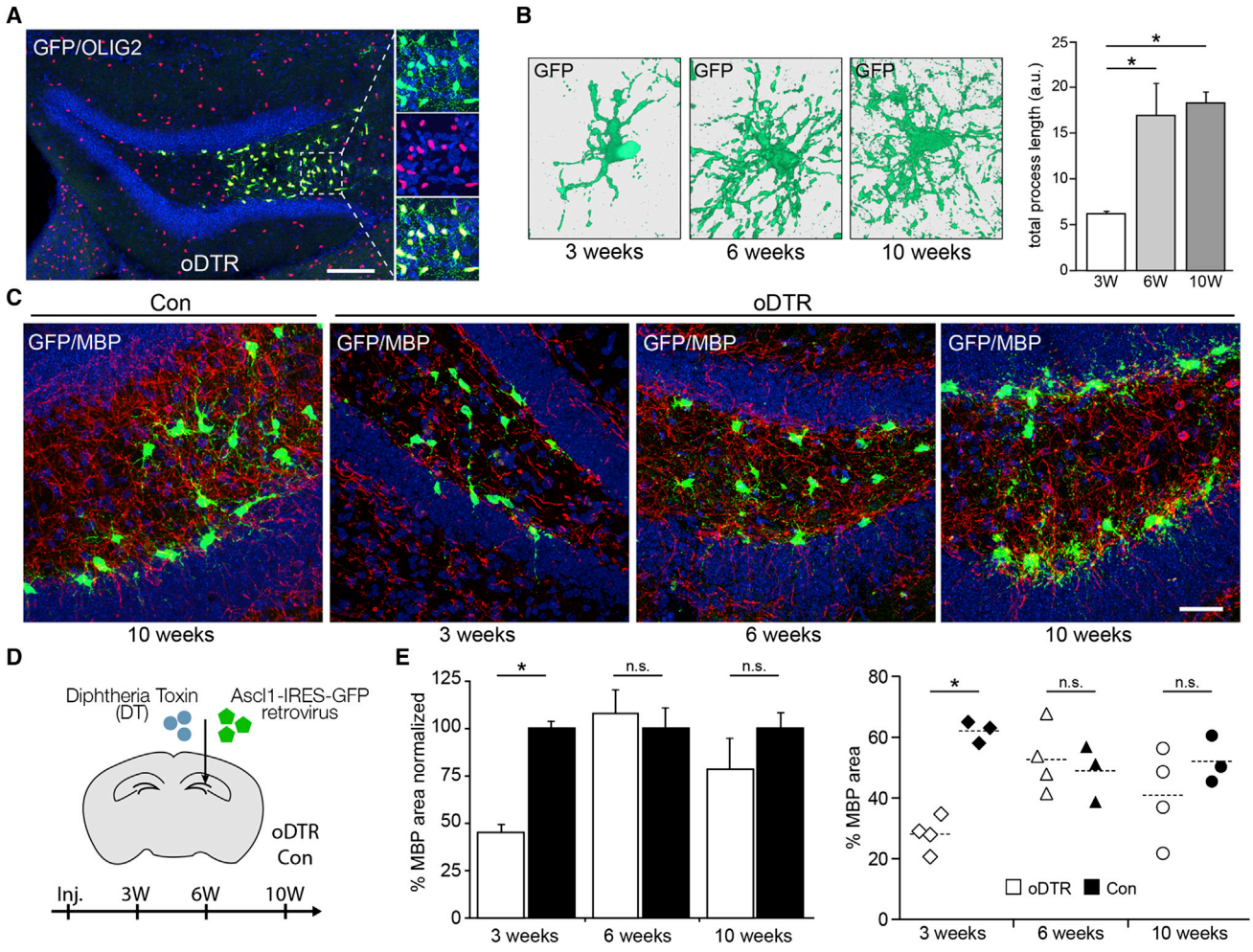

Figure 3. Directed Differentiation of Adult NSPCs Restores Hippocampal Myelination after Focal Ablation of Oligodendrocytes

(A) oDTR mice were co-injected with Ascl1-IRES-GFP expressing retroviruses and DT into the DG. Ascl1-overexpressing NSPCs (GFP positive, green) differentiate into oligodendrocytes (OLIG2 positive, red) and extend processes throughout the demyelinated hilus.

(B) The total process length of NSPC-derived oligodendrocytes was measured at 3, 6, and 10 weeks after injection into the hilus of oDTR mice. Example pictures show Ascl1-IRES-GFP expressing cells at different time points (GFP, 3D volumes rendered in green). Graph shows quantification of total process length (oDTR n = 4 for each time point, total of 48 cells).

(C) oDTR and Con mice were co-injected with Ascl1-IRES-GFP-expressing retroviruses (green) and DT into the DG, and MBP levels (red) were determined in the virus-infected regions within the hilus of the DG at 3, 6, and 10 weeks after the injections.

(D) Scheme describing the experimental setup.

(E) Graphs showing the quantification of hilar MBP levels in oDTR and Con mice at different time points. The left panel shows %MBP area normalized to control. The right panel shows measured %MBP areas. (oDTR n = 4 and Con n = 3, for each time point).

Error bars represent mean ± SEM. Scale bars represent 10 μm (A) and 40 μm (C). Nuclei were stained with DAPI (blue).

GFP+ and GFP− areas in oDTR mice (Figure S3). Over time, the NSPC-derived oligodendrocytes matured as determined by the expression of early (NG2), intermediate (CNPase), and late oligodendroglial lineage markers (MBP) at 3, 6, and 10 weeks after injury (Figures S4A–S4C). Furthermore, we observed an increase over time in oligodendrocyte process length of GFP-expressing cells (Figure 3B), a feature associated with maturing oligodendrocyte morphology.

To examine the remyelination potential of NSPC-derived oligodendrocytes, we analyzed the extent of demyelination in the virus-infected regions of the hilus at 3, 6, and 10 weeks after injury in oDTR and control mice (Figure 3D). At 3 weeks after injection, we observed a significant reduction in myelin levels in oDTR mice, similar to levels we observed in oDTR mice that were not co-injected with Ascl1-expressing virus (Figures 2C and 3C). However, at 6 and 10 weeks after injury, we observed a striking increase in myelin levels in the hilus of oDTR mice, reaching levels comparable to MBP levels observed in control mice that are not sensitive to DT-mediated demyelination (Figures 3C–3E). These data suggest that NSPC-derived oligodendrocytes mature within the DG niche and enhance remyelination after injury.

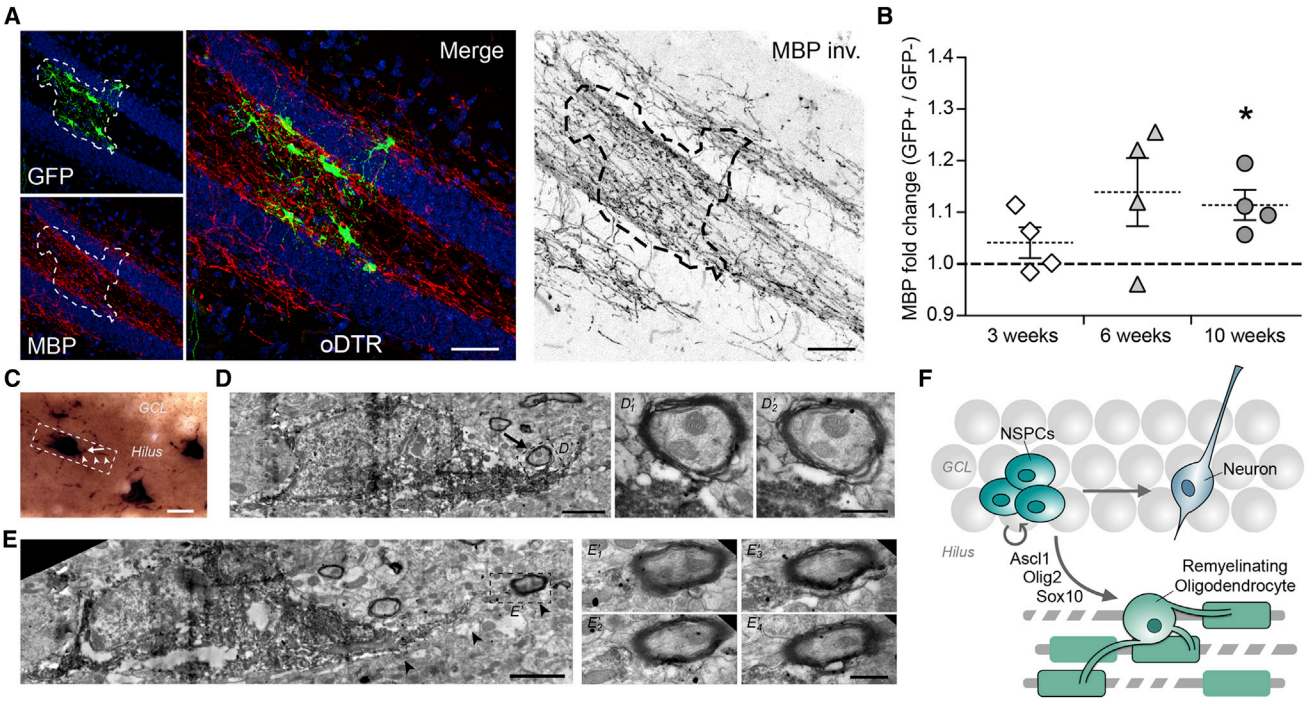

Figure 4. NSPC-Derived Oligodendrocytes Remyelinate Axons after Injury

(A) MBP levels were measured in neighboring GFP+ and GFP− regions in the hilus of oDTR mice 3, 6, and 10 weeks after co-injection of Ascl1-IRES-GFP and DT toxin. Representative image showing the maximum projection of inverted MBP staining with a dashed outline of the GFP+ region.

(B) Graph showing the fold change in %MBP area in GFP+ regions compared to GFP− regions. Note that when normalized to min (25%) and max (65%) MBP area, a fold change of 1.14 corresponds to a 34.7% increase in MBP in the GFP+ region (oDTR n = 4, for each time point).

(C) Electron microscopy analysis of remyelinating GFP+ oligodendrocytes in the hilus of oDTR mice 10 weeks after injury. A light microscopy image of the DAB stained GFP+ hilar cell that was analyzed by electron microscopy is shown. In addition to interactions close to the soma (arrow), processes extend over greater distances to ensheath axons (arrowheads).

(D) An electron micrograph showing a process extending from the soma of the immunoperoxidase-labeled GFP+ oligodendrocyte (arrow) and ensheathing the axon. In the corresponding high-magnification serial electron micrographs, note the processes forming the initial myelin wrap extending from the NSPC-derived, GFP-labeled cell.

(E) An electron micrograph showing a process extending from the soma of the immunoperoxidase-labeled GFP+ oligodendrocyte (arrowheads) toward an axon ~5 μm away. Serial electron micrographs (right) show the process approaching and eventually ensheathing the axon.

(F) Schematic representation of the main findings.

Error bars represent mean ± SEM. Scale bars represent 40 μm (A), 10 μm (C), 2 μm (D and E), and 0.5 μm (D′ and E′). Nuclei were stained with DAPI (blue).

NSPC-Derived Oligodendrocytes Myelinate Axons and Enhance Remyelination in the Adult Hippocampus after Injury

To determine the contribution of NSPC-derived oligodendrocytes to enhanced remyelination in oDTR mice after injury, we quantified MBP levels in GFP+ and GFP− hilar regions within the same animals. MBP levels were increased by up to 34% in regions containing Ascl1-IRES-GFP-expressing oligodendrocytes 6 and 10 weeks after injection (Figures 4A and 4B), indicating that fate-directed NSPC-derived oligodendrocytes are responsible for the recovery of myelination after injury. To directly show that these cells enwrap axons with myelin sheaths, we imaged Ascl1-IRES-GFP-expressing oligodendrocytes by immuno-electron microscopy. Strikingly, serial electron micrographs of Ascl1-IRES-GFP-expressing cells revealed the presence of myelin sheaths enwrapping axons in the hilus of oDTR mice 10 weeks after injury (Figures 4C–4E). Thus, these findings clearly demonstrate that directed differentiation of endogenous hippocampal NSPCs into myelinating oligodendrocytes enhances remyelination of the hippocampus after injury.

DISCUSSION

Previous studies have described the region-specific remyelination potential of cells derived from SVZ NSPCs, endogenous OPCs, and transplanted oligodendrocytic cells in distinct brain areas including the striatum and corpus callosum (Huang et al., 2011; Nakatani et al., 2013; Picard-Riera et al., 2002; Rafalski et al., 2013; Yang et al., 2013). We report that in the hippocampus, spontaneous remyelination was largely absent after demyelinating injury. To induce focal demyelination within the adult hippocampus, we established a novel model of genetic demyelination based on the oDTR mouse line allowing for DT-induced, focal ablation of oligodendrocytes. This approach is well suited for modeling oligodendrocyte loss and cell-replacement strategies as it does not rely on an immune response to induce

demyelination (Locatelli et al., 2012). After focal ablation of oligodendrocytes, we did not observe an increase in OPC numbers or spontaneous remyelination in the hippocampus. In addition, we found that neurogenesis levels were unchanged and that oligodendrogenesis was not spontaneously induced in the demyelinated hippocampus. These findings are supported by previous studies showing that remyelination after oligodendrocyte ablation does not occur equally throughout the brain (Locatelli et al., 2012; Pohl et al., 2011) and that certain regions, including the hippocampus, do not display significant levels of spontaneous remyelination after injury (Deverman and Patterson, 2012). This is of particular importance, as individuals suffering from demyelinating diseases such as multiple sclerosis or other diseases that impact myelination, such as traumatic brain injury and epilepsy, often show demyelination in temporal lobe regions and are affected by hippocampus-dependent cognitive deficits, underlining the need for therapies that promote remyelination within this brain region (Chambers and Perrone-Bizzozero, 2004; Hemanth Kumar et al., 2014; Meier et al., 2004; Noble, 2004; Benedict et al., 2009; Yang et al., 2009; Chiaravalloti and DeLuca, 2008; Geurts et al., 2007). Therefore directed differentiation of hippocampal NSPCs into oligodendrocytes that mature, stably integrate, and enhance remyelination in the lesioned DG represents a novel approach that harnesses the fate plasticity of endogenous NSPCs for glial cell replacement.

Notably, we show that directed differentiation of NSPCs into oligodendrocytes can be achieved within the adult hippocampus by retroviral overexpression of Ascl1, Olig2, or Sox10 TFs, which are known to play pivotal roles during brain development to induce oligodendrocyte lineage specification and maturation. Thus, the activation of this developmental genetic program in hippocampal NSPCs is sufficient to switch NSPC fate and to promote oligodendrogenesis. Future studies aiming to identify small molecules that can activate this transcriptional pathway in adult NSPCs will be of great interest to promote oligodendrocyte differentiation after injury without using retroviruses, as well as to develop strategies that promote migration of hippocampal NSPCs to other brain regions within the temporal lobe.

Importantly, we used several complementary approaches including electron microscopy to show that Ascl1-mediated fate conversion of hippocampal NSPCs into mature, myelinating oligodendrocytes enhances remyelination in the DG after injury (Figure 4F). Thus, the data shown here represent a proof of concept for the use of endogenous NSPCs for glial repair within the adult hippocampus.

EXPERIMENTAL PROCEDURES

Animals and Stereotactic Injections

All animal experiments were approved by the veterinary office of the Canton of Zurich, Switzerland. The DTR and MOG-Cre mouse lines crossed to generate oDTR mice have previously been described (Buch et al., 2005). Retroviruses were produced according to Zhao et al. (2006). Adult oDTR and Con mice (6–9 weeks) were injected in the DG with 0.5 ng DT (Calbiochem) and/or Ascl1-IRES-GFP-expressing retroviruses and/or control IRES-GFP-expressing retroviruses, diluted in a final volume of 1.5 μl PBS, according to a previously described protocol (Knobloch et al., 2013). Adult wild-type mice (6–9 weeks) were injected in the DG with Ascl1-IRES-GFP, Olig2-IRES-CFP, or Sox10-IRES-GFP expressing retroviruses diluted in 1.5 μl PBS. Animals were killed at 3, 6, or 10 weeks after injection as indicated. Brains were cut into 40-μm-thick free-floating sections, and stainings were performed as described previously (Knobloch et al., 2013). Detailed protocols can be found in the Supplemental Experimental Procedures.

Cell Culture

Hippocampal NSPCs were isolated from 6- to 8-week-old C57Bl/6 mice as previously described (Bracko et al., 2012). To analyze the effects of oligodendroglial TF overexpression on NSPC differentiation in vitro, adult NSPCs were infected with control IRES-GFP, Ascl1-IRES-GFP, Olig2-IRES-CFP, or Sox10-IRES-GFP expressing retroviruses and cultured for 48 hr before induction of differentiation. Differentiation was induced by growth factor withdrawal in DMEM/F12 supplemented with B27, heparin (5 m/ml), triiodo-L-thyronine (T3; 30 ng/ml, Sigma-Aldrich) and L-thyroxine (T4; 40 ng/ml, Sigma-Aldrich).

Image Analysis

Myelination quantification was performed on brain sections stained with MBP antibodies, and 20-μm sections of the DG were imaged by confocal microscopy (SP2, Leica). Maximum projections of the confocal stacks were thresholded using ImageJ (NIH), and the area of MBP staining was measured as a percentage value. For each injected animal, 63× images of three to four separate regions of the DG were analyzed and the %MBP area was averaged. For quantifications of virus-infected regions, GFP+ areas were traced in all images and the %MBP area quantification was restricted to the traced area. For co-localization experiments and cell number quantifications, confocal microscopy (SP2, Leica) was performed followed by analysis using ImageJ and Imaris (Bitplane). The NeuronJ plugin for ImageJ was used to measure total process length of GFP+ oligodendrocytes. Photoshop (Adobe) and Imaris were used for contrast enhancements, 3D rendering, and color adjustments. Detailed protocols can be found in the Supplemental Experimental Procedures.

Electron Microscopy

Ten weeks after injury, oDTR mice were transcardially perfused with fixative. Sections were then blocked and incubated overnight at 4°C in the primary antibody (rabbit α-GFP; 1:500; Invitrogen). Sections were washed and then incubated for 4 hr at 25°C with the secondary antibody (biotinylated goat α-rabbit; 1:200; Jackson Laboratories), followed by a 3,3-diaminobenzidine (DAB) peroxidase reaction (Vector Laboratories Kit, 10 min). After dehydration in ascending concentrations of ethanol and acetone, sections were lifted into resin (Durcupan ACM, Fluka) and left overnight (25°C). After locating Ascl1-IRES-GFP-expressing oligodendrocytes at the light-microscopic level, serial ultrathin sections (70-nm thick) were cut and collected onto Formvar-coated, single-slot copper grids. A Philips CM10 transmission electron microscope and a digital camera (Morada SIS, Olympus) were used to collect serial images of the labeled cells. Detailed protocols can be found in the Supplemental Experimental Procedures.

Statistical Analysis

Statistical analyses were performed using Excel (Microsoft). Two-sample t tests were used for all comparisons, except for Figure 4B, where a one-sample t test was used (significance was determined compared to a hypothetical mean of 1). Differences were considered significant at $p < 0.01$.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and four figures and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2015.05.024.

AUTHOR CONTRIBUTIONS

S.M.G.B. co-developed the concept, performed experiments, analyzed data, and co-wrote the paper. G.-A.P. and R.A.C.M. performed experiments. J.M. and N.T. performed EM experiments. B.B. contributed reagents and revised the manuscript. S.J. developed the concept and wrote the paper.

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Cell Reports

Supplemental Information

Programming Hippocampal Neural Stem/Progenitor Cells into Oligodendrocytes Enhances Remyelination in the Adult Brain after Injury

Simon M.G. Braun, Gregor-Alexander Pilz, Raquel A.C. Machado, Jonathan Moss, Burkhard Becher, Nicolas Toni, and Sebastian Jessberger

SUPPLEMENTAL FIGURES

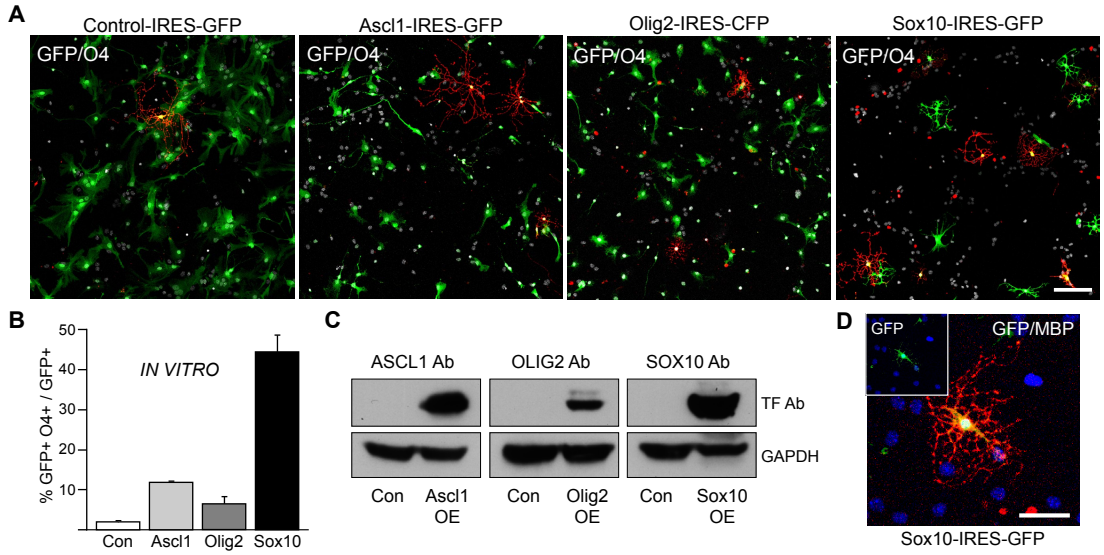

Figure S1 (related to Figure 1). Retroviral overexpression of Ascl1, Olig2 and Sox10 transcription factors in adult hippocampal NSPCs in vitro. (A) Adult hippocampal NSPC cultures were infected with control, Ascl1, Olig2 or Sox10 overexpressing retroviruses (with IRES-GFP for labeling) and differentiated for 7 days. Differentiated NSPCs were stained with O4 (red) and GFP (green) antibodies. (B) Graph shows percentage of GFP-expressing cells positive for the oligodendrocyte marker O4 (n=3 for each condition; >500 cells were analyzed per condition). (C) Western blot analysis of HEK-293T cells transfected with different viral constructs. ASCL1, OLIG2, SOX10 and GAPDH antibodies were used to show overexpression of TFs. (D) NSPC cultures infected with Sox10-IRES-GFP (green) overexpressing retroviruses differentiate into MBP (red) expressing oligodendrocytes.

Error bars represent mean ± SEM. Scale bars represent 40 μm. Nuclei were stained with DAPI (blue).

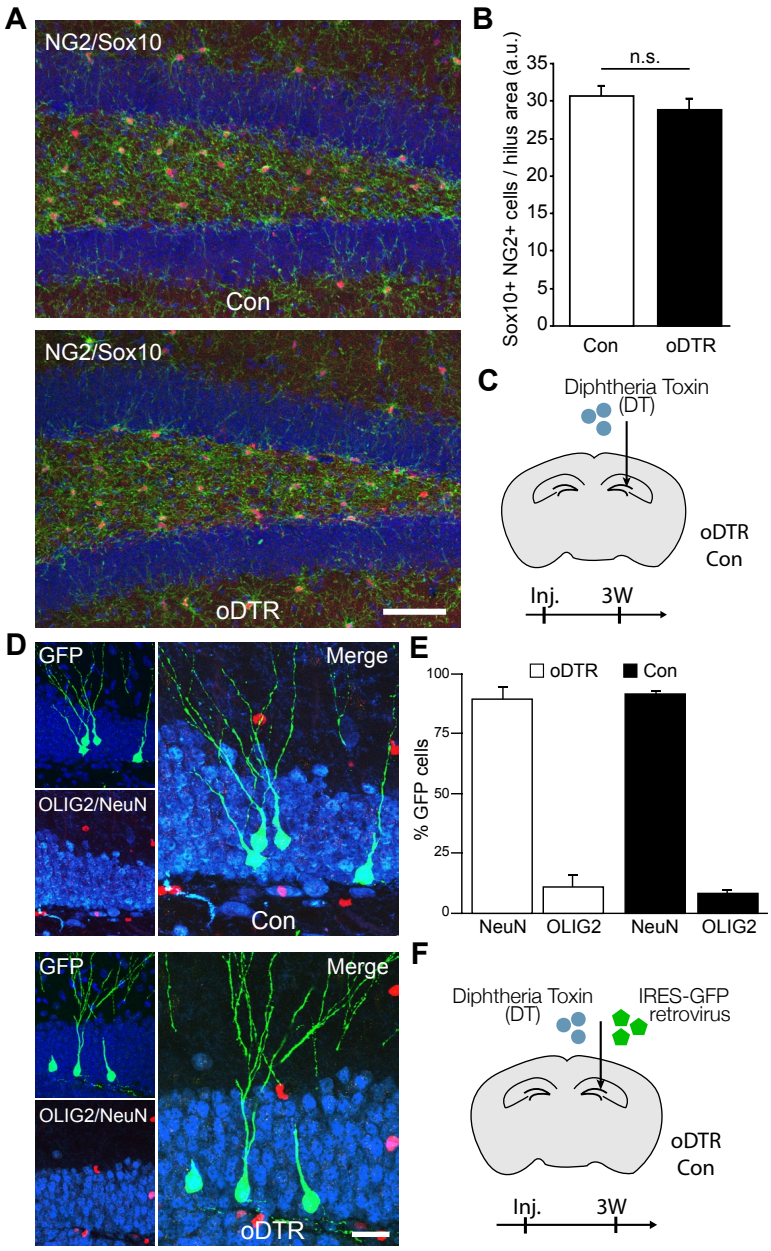

Figure S2 (related to Figure 2). (A) OPC numbers remain unchanged in the hilus 3 weeks after demyelination. oDTR and Con mice were injected with DT into the DG and OPC numbers (NG2+, green and SOX10+, red) in the hilus of the DG were counted 3 weeks after the injections. (B) Graph shows quantification of hilar NG2/SOX10+ OPC numbers in oDTR and Con mice (oDTR n=4 & Con n=3; >150 cells were phenotyped per condition). (C) Scheme describes the experimental setup.

(D) Fate of adult hippocampal NSPCs after focal demyelination in the DG. oDTR and Con mice were co-injected with control IRES-GFP expressing retroviruses (green) and DT into the DG. The neuronal (NeuN positive, blue) or oligodendroglial (OLIG2 positive, red) fate of the labeled NSPCs was determined 3 weeks after injection. (E) Graph shows quantification of GFP+/NeuN+ and GFP+/OLIG2+ cells in oDTR and Con mice. (oDTR n=3 & Con n=3; >100 cells were analyzed per condition). (F) Scheme describes the experimental setup.

Error bars represent mean ± SEM. Scale bars represent 60μm (A) and 20 μm (D). Nuclei were stained with DAPI (blue).

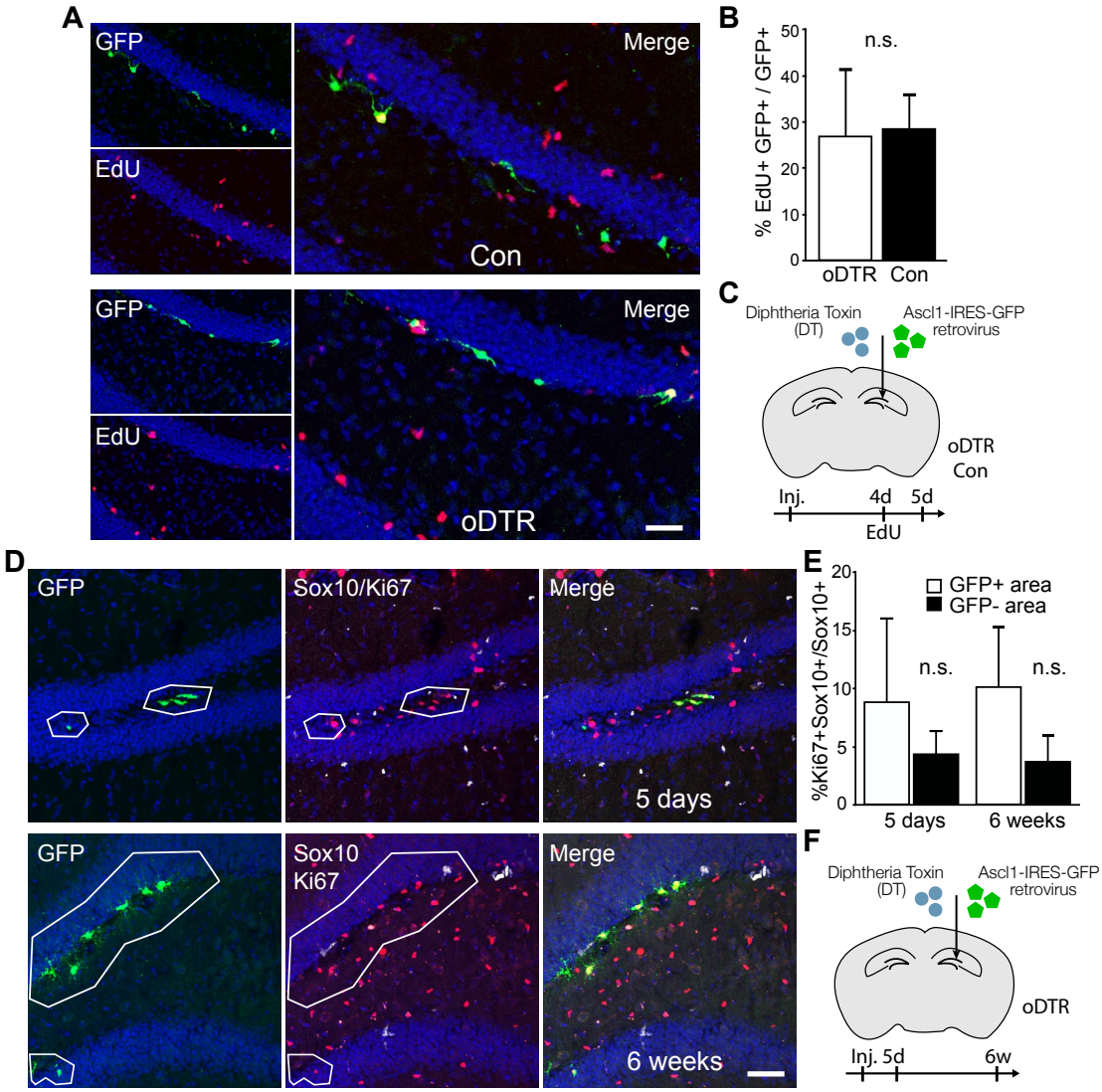

Figure S3 (related to Figure 3). Demyelination of the hippocampus does not alter proliferation levels of converted NSPCs and endogenous OPCs. (A) oDTR and Con mice were co-injected with Ascl1-IRES-GFP expressing retroviruses (green) and DT into the DG. The proliferation rate of converted NSPCs was measured following a 24h EdU (red) pulse performed 4 days post-injection. (B) Graph shows quantification of GFP+/EdU+ cells in oDTR and Con mice. (oDTR n=4 & Con n=3; >150 cells were analyzed per condition). (C) Scheme describes the experimental setup for panels A and B.

(D) oDTR mice were co-injected with Ascl1-IRES-GFP expressing retroviruses (green) and DT into the DG. Sox10+ (red) Ki67+ (white) OPC numbers were determined in GFP+ and GFP- areas within the hilus of the DG at 5 days and 6 weeks after the injections. (E) Graph shows quantification of Sox10+/Ki67+ cells in GFP+ and GFP- areas (5 days n=3 & 6 weeks n=4; >400 cells were analyzed per condition). (F) Scheme describes the experimental setup for panels D and E.

Error bars represent mean ± SEM. Scale bars represent 40 μm. Nuclei were stained with DAPI (blue).

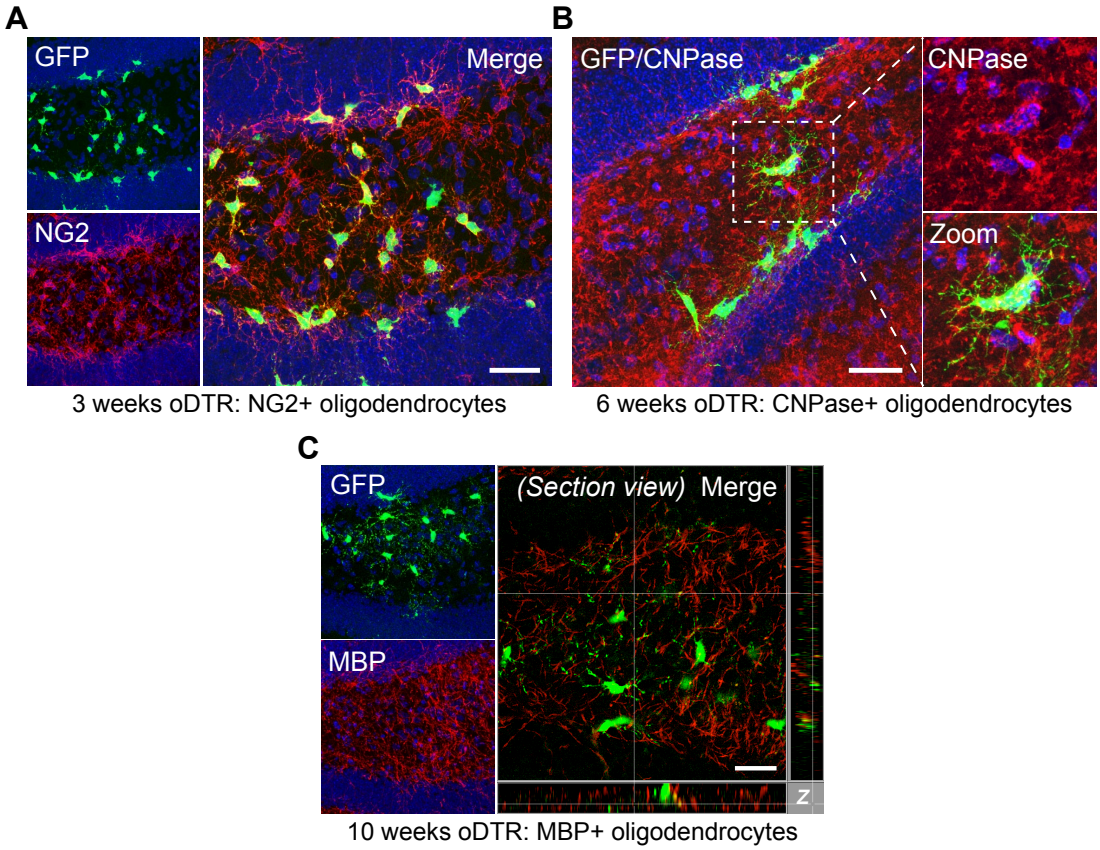

Figure S4 (related to Figure 4). Ascl1-overexpressing NSPCs differentiate into mature oligodendrocytes in the demyelinated hippocampus of oDTR mice. Ascl1-IRES-GFP overexpressing NSPCs express early (A) (NG2, red), intermediate (B) (CNPase, red) and late (C) (MBP, red) oligodendrocyte markers at 3, 6 and 10 weeks after demyelination in the hilus of oDTR mice. Right panel shows MBP/GFP staining as a section view; only partial co-labeling is observed in certain GFP processes due to myelin compaction, which limits access of GFP to the myelin cytoplasm.

Scale bars represent 40 μm. Nuclei were stained with DAPI (blue).

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Animals

All animal experiments were approved by the veterinary office of the Canton of Zurich, Switzerland. Mice were kept with littermates under a 12 hrs dark/light cycle in single ventilated cages and with ad libitum access to food and water. The DTR mice and MOG-Cre mice (Buch et al., 2005) were described before and genotyping was performed using previously described primers (Locatelli et al., 2012). Wild-type C57Bl/6J adult mice were purchased from Janvier Labs, France.

Retrovirus and DT injections

Coordinates for stereotactic injections from Bregma were as follows: a/p -2.0, m/l, ± 1.5, d/v -2.3 from skull. Adult oDTR and Con mice (6-9 weeks) were injected in the DG with 0.5 ng Diphtheria Toxin (DT, Calbiochem), and/or Ascl1-IRES-GFP expressing retroviruses, and/or control IRES-GFP expressing retroviruses, diluted in a final volume of 1.5 μL PBS. To measure proliferation rates, animals received 2 intraperitoneal injections of EdU (50 mg/kg, Sigma) 4 days after stereotactic injection of DT and retroviruses. Adult wild-type mice (6-9 weeks) were injected in the DG with Ascl1-IRES-GFP, Olig2-IRES-CFP or Sox10-IRES-GFP expressing retroviruses diluted in 1.5 μL PBS. Animals were killed at 3, 6 or 10 weeks post injection as indicated. For tissue collection, mice were given a lethal dose of Esconarkon (Streuli) and flushed transcardially with 0.9% sterile NaCl, followed by fixation with 4%

paraformaldehyde (PFA)/0.1 M phosphate buffer, pH 7.4. Brains were post-fixed in 4% PFA/0.1 M phosphate buffer overnight at 4°C followed by 30% sucrose/0.1 M phosphate buffer and stored at 4°C.

Retrovirus production

Retroviruses were produced as previously described (Zhao et al., 2006). In brief, ten 10 cm plates of confluent human embryonic kidney (HEK 293T) cells were transfected with retroviral constructs and packaging plasmids (pCMV-vsvg and pCMV-gp) using Lipofectamine 2000 (Life Technologies). Two days post transfection the virus containing cell culture media (100mL) was collected, filtered with a 0.22 µm Steritop filter (Millipore), and centrifuged at 19,400 rpm in an ultracentrifuge for 2 hrs at 4°C. The viral pellet was resuspended in 4 mL PBS and centrifuged a second time through a 20% sucrose cushion at 19,400 rpm for 2 hrs at 4°C. The final viral pellet was resuspended in 30 μL of PBS and used for subsequent infections.

Cell culture

Hippocampal NSPCs were isolated from 6-8 weeks old C57Bl/6 mice as previously described (Bracko et al., 2012). The resulting single cell suspension was cultured as sphere culture in DMEM/F12 (Life Technologies) medium supplemented with B27 (Life Technologies), human EGF (20 ng/ml, Peprotech) and human basic FGF-2 (20 ng/ml, Peprotech). Medium contained an antibiotic/antimycotic (Anti-Anti, Life Technologies). Cells were then plated on glass coverslips coated with Poly-L-ornithine (Sigma) and Laminin (Life

Technologies), and Heparin (5 mg/mL, Sigma) was added to the medium to obtain adherent cultures. All experiments were done in triplicates. To analyze the effects of oligodendroglial TF overexpression on NSPC differentiation in vitro, adult NSPCs were infected with control IRES-GFP, Ascl1-IRES-GFP, Olig2-IRES-CFP or Sox10-IRES-GFP expressing retroviruses, and cultured for 48 hrs, before induction of differentiation. Differentiation was induced by growth factor withdrawal in DMEM/F12 supplemented with B27, Heparin (5 m/mL), Triiodo-L-thyronine (T3, 30 ng/mL, Sigma) and L-Thyroxine (T4, 40 ng/mL, Sigma). Cells were fixed with 4% PFA for 15 min, 7 days after the onset of differentiation.

Immunohistochemistry

Brains were cut into 40 µm thick free-floating sections by snap freezing them with dry ice after mounting them on a microtome. For blocking, we used 1 x TBS containing 3% donkey serum and 0.2% Triton X-100 for 30 min. Primary antibody incubation was done overnight at 4°C in the concentrations specified below. Secondary antibodies (Jackson ImmunoResearch) were applied at 1:250 at RT for 1 hr. Cell nuclei were counterstained with 4-6-diamidino-2-phenylindole (DAPI, 1:5000, Sigma). For cell stainings, immunocytochemistry was performed as described above on cells fixed with 4% PFA for 15 min at 37°C. Antibodies used were mouse α-CNPase (Abcam, 1:400), goat α-DCX (Santa Cruz, 1:250), chicken α-GFP (Aves, 1:500), rabbit α-Ki67 (Novocastra, 1:1000), rat α-MBP (Serotec, 1:100), mouse α-NeuN (Millipore, 1:250), rabbit α-NG2 (Millipore, 1:250), α-O4 (Millipore, 1:100), rabbit α-OLIG2 (Millipore,

1:250), and goat α-SOX10 (Santa Cruz, 1:50). EdU stainings were performed before antibody incubation using the Click-iT EdU imaging kit (Invitrogen).

Image analysis

Myelination quantification was performed on brain sections stained with MBP antibodies and 20 µm sections of the DG were imaged by confocal microscopy (SP2, Leica). Maximum projections of the confocal stacks were thresholded using ImageJ (NIH) and the area of MBP staining was measured as a percentage value, rendering values independent of hilar area measured. For each injected animal, 63x images of the DG were analysed and the %MBP area was averaged. For quantifications of virus-infected regions, GFP+ areas were traced and the %MBP area quantification was restricted to the traced area. For co-localization experiments and cell number quantifications, confocal microscopy (SP2, Leica) was performed followed by analysis using ImageJ and Imaris (Bitplane). The NeuronJ plugin for ImageJ was used to measure total process length of GFP+ oligodendrocytes. Photoshop (Adobe) and Imaris were used for contrast enhancements, 3D rendering and color adjustments.

Electron Microscopy

Ten weeks after injury, oDTR mice were transcardially perfused with fixative (4% paraformaldehyde, w/v, in 0.1 M phosphate buffer; PB). Coronal sections (50 *µ*m) were cut, placed in cryoprotectant (20 min) and freeze-thawed in liquid nitrogen. Sections were then blocked with 0.5% bovine serum albumin

in 0.1 M PB (v/v, BSA-C, Aurion) and incubated overnight at 4°C in the primary antibody (rabbit α-GFP; 1:500; Invitrogen) in 0.1% BSA-C in 0.1 M PB. Sections were washed and then incubated for 4hr at 25°C with the secondary antibody (biotinylated goat α-rabbit; 1:200; Jackson Laboratories) in 0.1% BSA-C in 0.1 M PB. After PBS washes, a 90 min incubation in avidin biotin peroxidase complex (ABC Elite, Vector Laboratories) was followed by a 3,3-diaminobenzidine (DAB) peroxidase reaction (Vector Laboratories Kit, 10 min). Sections were then post-fixed overnight in 2.5% glutaraldehyde (v/v) in 0.1 M PB at 4°C. Sections were post-fixed in 1% osmium tetroxide in 0.1M PB (30 min) and dehydrated in ascending concentrations of ethanol and then acetone, before being lifted into resin (Durcupan ACM, Fluka) and left overnight (25°C). The sections were then placed on to microscope slides, coverslipped and the resin cured at 65°C for three days. Locations of peroxidase labeled, Ascl1-IRES-GFP expressing oligodendrocytes were identified at the light microscopic level and serial ultrathin sections (70 nm thick) were cut and collected on to Formvar-coated, single-slot copper grids (15 min lead stain for contrast). A Philips CM10 transmission electron microscope and a digital camera (Morada SIS, Olympus) were used to collect serial images of the labeled cells.

Western Blot

HEK 293T cells were transfected with retroviral constructs expressing Ascl1, Olig2 or Sox10 using Lipofectamine (Life Technologies). Cells were lysed and protein concentrations were determined by Bradford assay (Biorad). Proteins

were separated by SDS-PAGE electrophoresis, transferred to a nitrocellulose membrane (BioRad) and probed with primary antibodies, mouse α-Ascl1 (BD, 1:1000), rabbit α-Olig2 (Millipore, 1:1000), goat α-SOX10 (Santa Cruz, 1:1000) and mouse α-GAPDH (1:10000, HyTest) followed by HRP-conjugated secondary antibodies (Jackson ImmunoResearch) and bands were detected by chemiluminescence (Thermo Scientific).

Statistical analysis

Statistical analyses were performed using Excel (Microsoft). Two-sample t-tests were used for all comparisons; except for in Figure 4B we used a one-sample t-test (significance was determined compared to a hyp. mean of 1). Differences were considered significant at $p < 0.01$.

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