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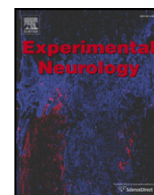

Short Communication

Mobilization of progenitors in the subventricular zone to undergo oligodendrogenesis in the Theiler's virus model of multiple sclerosis: Implications for remyelination at lesions sites

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ABSTRACT

Remyelination involves the generation of new myelin sheaths around axons, as occurs spontaneously in many multiple sclerosis (MS) lesions and other demyelinating diseases. When considering repairing a diseased brain, the adult mouse subventricular zone (SVZ) is of particular interest since the stem cells in this area can migrate and differentiate into the three major cell types in the central nervous system (CNS). In Theiler's murine encephalomyelitis virus-induced demyelinating disease (TMEV-IDD), we assessed the relative contribution of the SVZ to the remyelination in the corpus callosum at preclinical stages in this MS model. CNPase, MBP and Luxol Fast Blue staining revealed prominent demyelination 35 days post-infection (dpi), concomitant with a strong staining in GFAP$^+$ type B astrocytes in the SVZ and the increased proliferation in this area. The migration of oligodendrocyte progenitors from the SVZ contributed to the remyelination observed at 60 dpi, evident through the number of APC$^+$/BrdU$^+$ mature oligodendrocytes in the corpus callosum of infected animals. These data suggest that the inflammation induced by the Theiler's virus not only provokes strong preclinical demyelination but also, it is correlated with oligodendrocyte generation in the adult SVZ, cells that along with resident progenitor cells contribute to the prompt remyelination observed in the corpus callosum.
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The pathological loss of myelin in diseases like multiple sclerosis (MS) is usually followed by a phenomenon of remyelination, in which oligodendrocytes synthesize new myelin sheaths to cover the exposed axons in the adult central nervous system (CNS; Chari, 2007). This remyelination is not only important to restore saltatory conduction but it also protects axons from different insults, thereby limiting the clinical disability associated to demyelinating diseases (Chandran et al., 2008). In such pathological situations, much attention has been paid to the mammalian subventricular zone (SVZ) as a potential source of cells that can replace those that are lost following insult or injury. The stem cells in this niche support long-distance migration (Kim and Szleze, 2008), and they can be activated in MS patients to promote gliogenesis (Nait-Oumesmar et al., 2007). Although NG2$^+$ precursor cells are the first to react to demyelination, displaying the highest rate of proliferation (Watanabe et al., 2002), the relative contribution of the SVZ in the response to inflammatory demyelination induced by Theiler's virus infection and oligodendrogenesis has yet to be addressed. In the present study, we investigated the behavior of the SVZ in Theiler's murine encephalomyelitis virus-induced demyelinating disease (TMEV-IDD). We show that demyelination occurs in the corpus callosum during the preclinical phase of the disease in this viral model of MS, which is accompanied by a mobilization of progenitors in the SVZ to undergo oligodendrogenesis. We observe an increase in the proliferative rate in this area, with no activation of NG2$^+$ precursors but strong GFAP$^+$ type B astrocytes staining close to the lateral brain ventricles. Finally, we show for the first time that Theiler's infection enhances the mobilization of SVZ progenitor cells to the surrounding demyelinated corpus callosum, generating mature APC$^+$ oligodendrocytes.

Materials and methods

Animals and Theiler's virus infection

TMEV-IDD-susceptible female SJL/J mice from our in-house colony (Cajal Institute, Madrid) were maintained on a 12 h light/dark cycle with *ad libitum* access to food and water. Four-week-old mice were inoculated intracerebrally into the right hemisphere with 2×10^6 plaque forming units (pfu) of the Daniels (DA) strain of TMEV kindly provided by Dr. Moses Rodriguez, in 30 μl of Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal calf serum (FCS), as described

Abbreviations: EAE, experimental autoimmune encephalomyelitis; CNS, central nervous system; LPC, lysophosphatidyl-choline; MS, multiple sclerosis; SVZ, subventricular zone; TMEV-IDD, Theiler's murine encephalomyelitis virus-induced demyelinating disease.

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previously (Lledo et al., 1999). Sham uninfected mice were administered 30 μl of DMEM + 10% FCS alone. Animals were handled in accordance with the European Union animal care guidelines (2010/63/EU).

Proliferation and cellular migration protocols

To study proliferation, Sham and TMEV mice received two intraperitoneal (i.p.) injections of BrdU (5 mg/kg: Sigma) at 2 h intervals on day 35 post-infection (dpi), and they were sacrificed 2 h after the last injection. To study SVZ cell migration, Sham and TMEV mice received four i.p. injections of BrdU (5 mg/kg) at 2 h intervals on 2 days at 35 dpi. The mice were maintained in our animal house for 15 days and they were sacrificed on day 45 pi. This tracing protocol has been described previously to label cells restricted to the SVZ and the rostral migratory stream (Picard-Riera et al., 2002). To study myelin proteins in the corpus callosum, animals were maintained until day 60 pi based on previous studies of the laboratory (Mecha et al., 2013).

Tissue processing

Mice were anesthetized with pentobarbital (50 mg/kg body weight, i.p.) and perfused with saline. The animal's brain were fixed overnight in 4% paraformaldehyde prepared in 0.1 M phosphate buffer (PB), and cryoprotected in 15% sucrose solution in 0.1 M PB, and then in a 30% sucrose solution. Coronal cryostat sections (30 μm thick) were obtained and processed for immunohistochemistry.

Immunohistochemistry

Free-floating brain sections were washed three times for 10 min with 0.1 M PB and after inhibiting the endogenous peroxidase, they were blocked for 1 h at room temperature (RT) in blocking buffer [0.2% Triton X-100 and 5% normal goat serum (NGS); Vector Laboratories, CA, USA]. The sections were then incubated overnight at 4 °C in blocking buffer containing the antibody diluted 1:500 against GFAP (generated in mouse, Sigma–Aldrich, MO, USA), MBP (generated in mouse, Millipore, MA, USA) or CNPase (generated in mouse, Sigma–Aldrich, MO, USA). The following day, the sections were rinsed three times for 10 min with PB + 0.2% Triton X-100 and they were then incubated for 1 h with a biotinylated goat-anti mouse antibody (Vector Laboratories, CA, USA). For immunostaining with DAB, the sections were incubated for 1 h with an avidin–biotin–peroxidase (ABC) complex (Vector Laboratories, CA, USA) and finally with the chromogen 3,3′ diaminobenzidine tetrahydrochloride (DAB; Sigma–Aldrich, MO, USA). For immunofluorescence, sections were incubated with a fluorescent secondary antibody conjugated with Alexa® fluorophore (1:500; Molecular Probes, OR, USA) in blocking buffer, washed and mounted. After staining, the sections were dehydrated, cleared with xylene and coverslipped. In all cases, the specificity of the staining was confirmed by omitting the primary antibody.

Immunofluorescence for proliferative and recruited cells

Sections were first incubated in HCl 2 N for 2 h at RT and they were then washed four times in PB and blocked for 1 h at RT in blocking buffer. The sections were then incubated for four days at 4 °C in blocking buffer containing the antibody against BrdU (1:200; generated in rat, Abcam, Cambridge, UK) and NG2 (1:200, generated in rabbit, Millipore, MA, USA) or APC (1:500 generated in mouse; Sigma-Aldrich, MO, USA). Subsequently, the sections were rinsed four times in PB and then incubated with the fluorescent secondary antibody conjugated with Alexa® fluorophore (1:500; Molecular Probes, OR, USA) in blocking buffer. Finally, the secondary antibody was washed with PB and the slides were mounted with PB:glycerol.

Analysis and counting of proliferative and recruited cells

The mean number of total $BrdU^+$ cells, $NG2^+/BrdU^+$, and $APC^+/BrdU^+$ labeled cells was determined by manual counting positive cells with the cell counter of Image J software (designed by National Institutes of Health) at the level of the SVZ in the lateral ventricles (for proliferative cells) or the corpus callosum (for proliferative $NG2^+$ cells and for APC^+ recruited cells) of eight consecutive sections. For each brain structure, the data are expressed as the number of cells/mm^2 as the mean of at least 4 mice per experimental group.

Microscopy and image analysis of MBP staining

Immunofluorescent images were acquired on a Leica TCS SP5 confocal microscope and a Zeiss Axiocam high resolution digital color camera was used to record the immunohistochemistry images. Individual images of 8 sections acquired from at least 4 animals per group were analyzed quantifying the intensity of MBP staining in the corpus callosum using ImageJ software as detailed as follows: first, we defined the regions of interest (corpus callosum) with the freehand tool; second, we split the channels of the selected area, obtaining one image per channel; third, we established a constant threshold of intensity in Sham animals to apply it to all the experimental groups; and four, we measured the staining intensity of all images within the experiments. The threshold intensity was maintained constant during the comparison and measuring all experimental and control images.

Data analysis

All the data are expressed as the mean ± SEM. One-way ANOVA followed by the Bonferroni post-hoc test, or Kruskal–Wallis ANOVA followed by Mann–Whitney U test was used to determine the statistical significance. The level of significance was set at $p \leq 0.05$.

Results

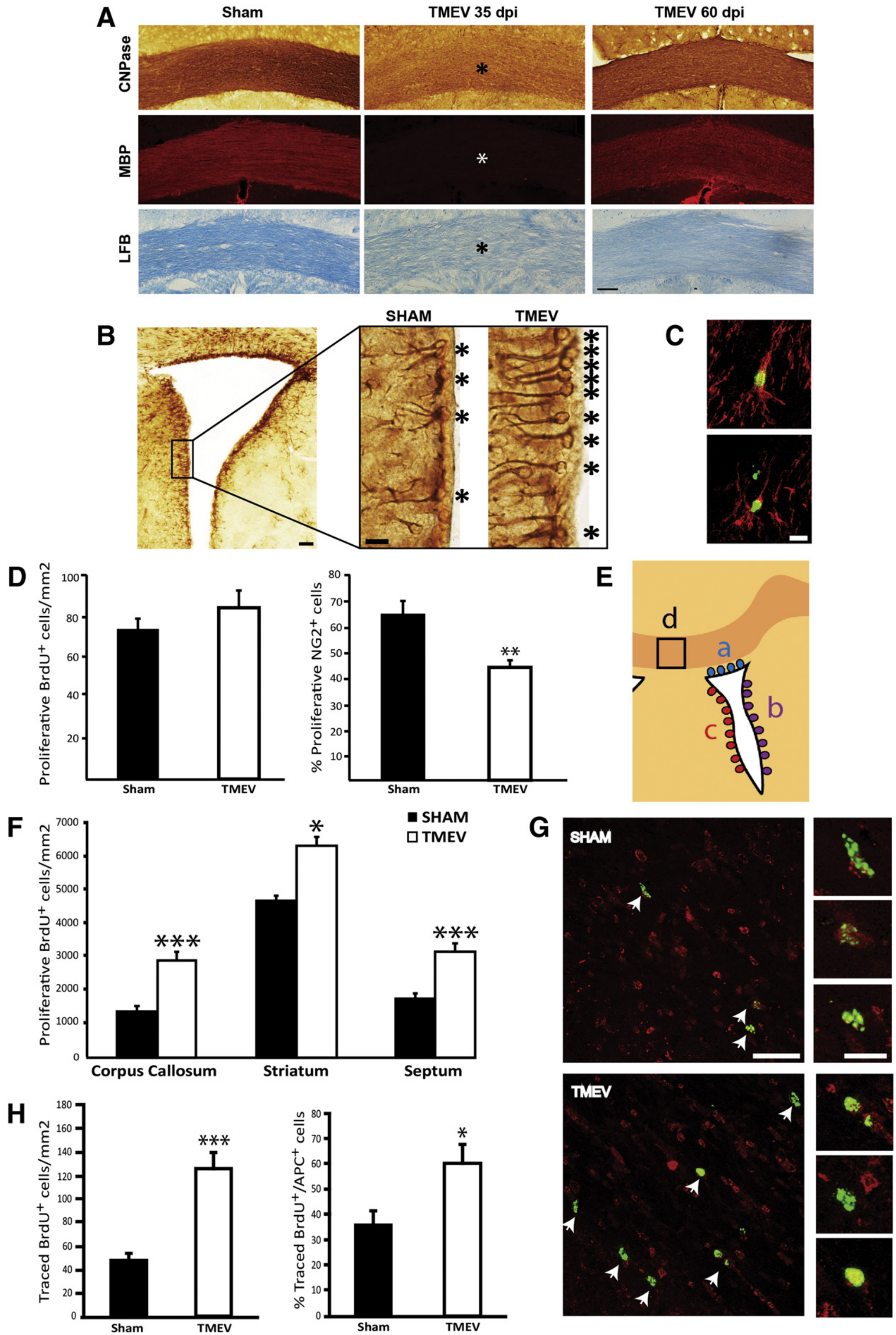
Demyelination and remyelination in the corpus callosum of TMEV-infected mice

We previously described presumptive remyelination in the motor cortex and brainstem of TMEV-IDD mice (Mecha et al., 2013). Here, we examined typical myelin markers in the corpus callosum, such as CNPase (non-compact myelin marker), MBP (compact myelin marker), and Luxol Fast Blue (LFB) staining, which revealed strong demyelination in this structure 35 dpi with TMEV (Fig. 1A). Notably, the loss of these markers was followed by an increase in their expression at 60 dpi, as measured with the quantification of MBP staining in this zone at 35 dpi (5.6 ± 2.19% staining intensity, $p \leq 0.001$ vs Sham) and 60 dpi (53.66 ± 11.47% staining intensity, $p \leq 0.01$ vs TMEV 35 dpi) suggesting a remyelination process in the damaged brain of infected animals long before any motor symptoms are evident.

$GFAP^+$ type B astrocytes in the lateral ventricles: SVZ mobilization

We used GFAP as a marker for astrocytes and stem cells to assess the relative contribution of the SVZ of TMEV-IDD mice (Alvarez-Buylla and Garcia-Verdugo, 2002). At 35 dpi we found GFAP staining of cells that were in contact with the ventricular surface of this region, which had a large soma and a long apical process running parallel to the ependymal layer (Supplemental Fig. 1A). These cells were type B astrocytes, previously described SVZ stem cells (Supplemental Fig. 1B) that can generate $NG2^+$ progenitor cells that express oligodendrocyte lineage transcription factor 2 (Olig2), and can be the origin of mature myelinating oligodendrocytes (Menn et al., 2006).

Besides, the strong staining of $GFAP^+$ cells with a type B astrocyte morphology close to the wall of the lateral ventricles in infected mice


A
Sham
TMEV 35 dpi
TMEV 60 dpi
CNPase
MBP
LFB
B
SHAM
TMEV
C
D
Proliferative BrdU+ cells/mm2
% Proliferative NG2+ cells
Sham
TMEV
E
a
b
c
d
F
Proliferative BrdU+ cells/mm2
SHAM
TMEV
Corpus Callosum
Striatum
Septum
G
SHAM
TMEV
H
Traced BrdU+ cells/mm2
% Traced BrdU+/APC+ cells
Sham
TMEV

suggested a mobilization the SVZ in TMEV-IDD mice at 35 dpi (Fig. 1B). Since adult oligodendrocyte progenitor cells (OPCs) express NG2 and they can divide and replace mature oligodendrocytes (McTigue and Tripathi, 2008), we evaluated whether these cells might have been activated in response to demyelination. $NG2^+$ activated cells morphologically enlarge their bodies, their processes become shorter and thicker, and there is an up-regulation of NG2 immunoreactivity that precedes an increase in proliferation within the lesion site (Polito and Reynolds, 2005). In our model, we did not detect any morphological change in $NG2^+$ cells in the TMEV infected mice at the level of the SVZ (Supplemental Fig. 1C) the corpus callosum (Fig. 1C). However, at the corpus callosum we observed the same proliferative ratio both in Sham and TMEV animals (Fig. 1D) and less proliferative $NG2^+$ cells at 35 dpi (64.91 ± 6.19% of total $BrdU^+$ cells in Sham, and 44.46 ± 3.42% of total $BrdU^+$ cells in TMEV; $p \leq 0.01$).

Proliferation in the SVZ, and migration towards the corpus callosum in TMEV- infected mice

To quantify proliferation in the SVZ we administered BrdU at 35 dpi, which was incorporated into cells in the lateral ventricles in contact with the corpus callosum, striatum and septum. With respect to the control mice there was a significant increase in the number of $BrdU^+$ cells in infected animals in all three of these areas (Figs. 1E, F). Moreover, a short BrdU pulse at 35 dpi produced an increase in the number of cells that incorporated BrdU in the corpus callosum of the infected animals at 45 dpi (Figs. 1E, G, H), suggesting that the OPCs had migrated from the SVZ towards the sites of demyelinaton. Indeed, double staining for BrdU and APC, a mature oligodendrocyte marker, demonstrated a significant increase in the number of $BrdU^+/APC^+$ cells in TMEV-IDD mice, which could at least partially explain the enhanced staining for myelin markers at 60 dpi.

Discussion

Lesions in chronically infected susceptible mice are associated with minimal spontaneous myelin repair (Bieber et al., 2005; Dal Canto and Lipton, 1975; Rodriguez and Lennon, 1990). Here we suggest that there is an early regenerative response in the SVZ to the brain initial damage, but this response is suppressed by the chronic inflammatory environment in TMEV-IDD mice, as CNS demyelination becomes prominent in the latter stages of the disease (Mecha et al., 2013). In particular, progressive motor deficits associated with spinal cord lesions are from 90 to 120 dpi onwards. In this study, we show that there is a spontaneous remyelination in the corpus callosum at 60 dpi, which has been previously described in the motor cortex and brainstem of TMEV-IDD mice in our laboratory. Moreover, the demyelination phase that occurs at 35 dpi, long before motor deficits are detected, is accompanied by strong staining of $GFAP^+$ type B astrocytes close to the lateral ventricles. These cells are known to exist close to the ventricular surface of the SVZ, possessing a single cilium and with a long basal process running parallel to the ependymal layer (Mirzadeh et al., 2008; Shen et al., 2008). Our results highlight the contribution of SVZ in the remyelination process, supported by the increased proliferation ratio of this germinative niche as shown by the BrdU incorporation analysis. However, the relative contribution of $NG2^+$ precursors to the remyelination process cannot be discarded, since they proliferate at 35 dpi although no morphological changes were observed at this time.

The participation of the SVZ to remyelination has been studied in other experimental mouse models of demyelination, like lysophosphatidyl-choline (LPC) injection, EAE or lysolecithin lesion (Menn et al., 2006; Picard-Riera et al., 2002). It also appears that the mobilization of the SVZ generates precursors in these models, suggesting they could also differentiate into mature oligodendrocytes and remyelinate demyelinated areas close to the lateral ventricles. Indeed, more Dcx^+ cells in the SVZ have been seen during preclinical phases in the TMEV-IDD model, which is not provoking an increase in the number of neuroblasts in the corpus callosum of these animals (Goings et al., 2008). In the TMEV-IDD model, such a phenomenon could be explained if these cells adopt an oligodendroglial fate and remyelinate the demyelinated areas, as we show by the increase in $BrdU^+/APC^+$ cells in the corpus callosum of TMEV-IDD model at a preclinical stage.

In summary, Theiler's virus infection of susceptible mice induces early demyelination of the corpus callosum that is accompanied by a mobilization of the SVZ which is later translated into increased progenitor cell proliferation in this area. These cells migrate towards the corpus callosum where they appear to be responsible together with local $NG2^+$ precursors for the remyelination observed later in these animals.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.expneurol.2013.10.011.

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References

Alvarez-Buylla, A., Garcia-Verdugo, J.M., 2002. Neurogenesis in adult subventricular zone. J. Neurosci. 22 (3), 629–634.
Bieber, A.J., Ure, D.R., Rodriguez, M., 2005. Genetically dominant spinal cord repair in a murine model of chronic progressive multiple sclerosis. J. Neuropathol. Exp. Neurol. 64 (1), 46–57.
Chandran, S., Hunt, D., Joannides, A., Zhao, C., Compston, A., Franklin, R.J.M., 2008. Myelin repair: the role of stem cells and precursor cells in multiple sclerosis. Philos. Trans. R. Soc. Lond. B Biol. Sci. 363 (1489), 171–183.

Fig. 1. Mobilization and differentiation of oligodendrocyte progenitors from the SVZ into the corpus callosum of TMEV-IDD mice. (A) Representative microphotographs of coronal brain sections (30 μm) immunostained for CNPase and MBP, or stained with LFB, showing a decrease in the intensity of staining at 35 dpi and an increase at 60 dpi. These changes were associated with remyelination in preclinical stages. Scale bar = 100 μm. (B) Microphotographs of coronal brain sections (30 μm) showing the lateral ventricles of infected mice at 35 dpi immunostained for GFAP, showing that TMEV infection induces at 35 dpi a strong staining of $GFAP^+$ cells close to the wall of the lateral ventricles when compare to Sham animals, scale bar = 200 μm for magnification, 100 μm for inserts. (C) Immunohistochemical staining of proliferative (BrdU, green) and $NG2^+$ (red) cells in the corpus callosum show that there are no morphological changes in this progenitor cells at 35 dpi, scale bar = 100 μm. (D) The proliferative ratio of this area does not change between groups at 35 dpi, although the percentage of $NG2^+/BrdU^+$ cells is decreased in TMEV infected mice. Statistical significance was determined by one-way ANOVA followed by Bonferroni post-hoc test: $^{**}p \leq 0.01$ vs Sham animals. (E) Schematic representation of the SVZ at 35 dpi, showing the areas of analysis of proliferative $BrdU^+$ cells in the lateral ventricle close to the corpus callosum (a), striatum (b), septum (c), and traced $BrdU^+$ cells in the corpus callosum (d). (F) Quantification of the number of $BrdU^+$ cells per mm^2 at 35 dpi reveals an increase in the proliferation of cells in the wall of the SVZ close to the corpus callosum, striatum or septum in TMEV-IDD mice. Statistical significance was determined using a Kruskal–Wallis ANOVA test followed by the Mann–Whitney U test: $^{*}p \leq 0.05$; $^{***}p \leq 0.001$ vs Sham animals. (G) Representative microphotographs of coronal brain sections of the corpus callosum at 45 dpi (30 μm) immunostained for BrdU (green) and APC (red), scale bar = 50 μm, 10 μm (inserts). (H) Quantification of the number of $BrdU^+$ (left) and $BrdU^+/APC^+$ cells (right) per mm^2 shows an increase at 45 dpi in the number of cells from the SVZ that migrate to the corpus callosum in TMEV-IDD mice at preclinical stages. Statistical significance was determined using a Kruskal–Wallis ANOVA followed by the Mann–Whitney U test: $^{*}p \leq 0.05$; $^{***}p \leq 0.001$ vs Sham animals.

Chari, D.M., 2007. Remyelination in multiple sclerosis. Int. Rev. Neurobiol. 79, 589–620.

Dal Canto, M.C., Lipton, H.L., 1975. Primary demyelination in Theiler´s virus infection. An ultrastructural study. Lab. Investig. 33 (6), 626–637.

Goings, G.E., Greisman, A., James, R.E., Abram, L.K.F., Begolka, W.S., Miller, S.D., Szele, F.G., 2008. Hematopoietic cell activation in the subventricular zone after Theiler´s virus infection. J. Neuroinflammation 5, 44.

Kim, Y., Szleze, F.G., 2008. Activation of the subventricular zone stem cells after neuronal injury. Cell Tissue Res. 331, 337–345.

Lledo, A., Borrell, J., Guaza, C., 1999. Dexamethasone regulation of interleukin-1-receptors in the hippocampus of Theiler´s virus-infected mice: effects on virus-mediated demyelination. Eur. J. Pharmacol. 372 (1), 75–83.

McTigue, D.M., Tripathi, R.B., 2008. The life, death and replacement of oligodendrocytes in the adult CNS. J. Neurochem. 107, 1–19.

Mecha, M., Carrillo-Salinas, F.J., Mestre, L., Feliú, A., Guaza, C., 2013. Viral models of multiple sclerosis: neurodegeneration and demyelination in mice infected with Theiler´s virus. Prog. Neurobiol. 101–102, 46–64.

Menn, B., Garcia-Verdugo, J.M., Yaschine, C., Gonzalez-Perez, O., Rowitch, D., Alvarez-Buylla, A., 2006. Origin of oligodendrocytes in the subventricular zone of the adult brain. J. Neurosci. 26 (30), 7907–7918.

Mirzadeh, Z., Merkle, F.T., Soriano-Navarro, M., García-Verdugo, J.M., Alvarez-Buylla, A., 2008. Neural stem cells confer unique pinwheel architecture to the ventricular surface in neurogenic regions of the adult brain. Cell Stem Cell 3 (3), 265–278.

Nait-Oumesmar, B., Picard-Riera, N., Kerninon, C., Decker, L., Seilhean, D., Höglinger, G.U., Hirsch, E.C., Reynolds, R., Evercooren, A.B.V., 2007. Activation of the subventricular zone in multiple sclerosis: evidence for early glial progenitors. Proc. Natl. Acad. Sci. U. S. A. 104 (11), 4694–4699.

Picard-Riera, N., Decker, L., Delarasse, C., Goude, K., Nait-Oumesmar, B., Liblau, R., Pham-Dinh, D., Evercooren, A.B., 2002. Experimental autoimmune encephalomyelitis mobilizes neural progenitors from the subventricular zone to undergo oligodendrogenesis in adult mice. Proc. Natl. Acad. Sci. U. S. A. 99 (20), 13211–13216.

Polito, A., Reynolds, R., 2005. NG2-expressing cells as oligodendrocyte progenitors in the normal and demyelinated adult central nervous system. J. Anat. 207 (6), 707–716.

Rodriguez, M., Lennon, V.A., 1990. Immunoglobulins promote remyelination in the central nervous system. Ann. Neurol. 27 (1), 12–17.

Shen, Q., Wang, Y., Kokovay, E., Lin, G., Chuang, S.M., Goderie, S.K., Roysam, B., Temple, S., 2008. Adult SVZ stem cells lie in a vascular niche: a quantitative analysis of niche cell-cell interactions. Cell Stem Cell 3 (3), 289–300.

Watanabe, M., Toyama, Y., Nishiyama, A., 2002. Differentiation of proliferated NG2-positive glial progenitor cells in a remyelinating lesion. J. Neurosci. Res. 69 (6), 826-36.