Two-photon imaging of remyelination of spinal cord axons by engrafted neural precursor cells in a viral model of multiple sclerosis

Milton L. Greenberg, Jason G. Weinger, Melanie P. Matheu, Kevin S. Carbajal, Ian Parker, Wendy B. Macklin, Thomas E. Lane, Michael D. Cahalan

*Department of Physiology and Biophysics, Institute for Immunology, Department of Molecular Biology and Biochemistry, Sue and Bill Gross Stem Cell Center, Department of Neurobiology and Behavior, Multiple Sclerosis Research Center, University of California, Irvine, CA 92697; and Department of Cell and Developmental Biology, University of Colorado School of Medicine, Aurora, CO 80045

Contributed by Michael D. Cahalan, April 18, 2014 (sent for review February 18, 2014)

Neural precursor cells (NPCs) offer a promising approach for treating demyelinating diseases. However, the cellular dynamics that underlie transplanted NPC-mediated remyelination have not been described. Using two-photon imaging of a newly developed ventral spinal cord preparation and a viral model of demyelination, we describe the motility and intercellular interactions of transplanted mouse NPCs expressing green fluorescent protein (GFP) with damaged axons expressing yellow fluorescent protein (YFP). Our findings reveal focal axonal degeneration that occurs in the ventral side of the spinal cord within 1 wk following intracranial instillation with the neurotropic JHM strain of mouse hepatitis virus (JHMV). Axonal damage precedes extensive demyelination and is characterized by swelling along the length of the axon, loss of YFP signal, and transected appearance. NPCs engrafted into spinal cords of JHMV-infected mice exhibited diminished migration velocities and increased proliferation compared with transplanted cells in noninfected mice. NPCs preferentially accumulated within areas of axonal damage, initiated direct contact with axons, and subsequently expressed the myelin proteolipid protein gene, initiating remyelination. These findings indicate that NPCs transplanted into an inflammatory demyelinating microenvironment participate directly in therapeutic outcome through the wrapping of myelin around damaged neurons.

Significance

Stem cell transplantation has emerged as a promising cell-based therapy for the treatment of demyelinating diseases such as multiple sclerosis (MS). This study provides the first real-time imaging of transplanted stem cell-mediated remyelination in a mouse model of MS. Whereas current treatments solely delay disease progression, transplanted stem cells actively reverse clinical disease in animal models. Using two-photon microscopy and viral-induced demyelination, we describe a technique to visualize cellular migration and remyelination in the mouse spinal cord. Transplanted neural precursor cells physically wrap damaged axons with newly formed myelin, preserving axonal health.


The authors declare no conflict of interest.

1M.L.G. and J.G.W. contributed equally to this work.

2Present address: Diabetes Center, University of California, San Francisco, CA 94143.

3Present address: Department of Neurology, University of Michigan School of Medicine, Ann Arbor, MI 48109.

4Present address: Division of Microbiology and Immunology, Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT 84132.

5To whom correspondence may be addressed. E-mail: mcahalan@uci.edu or tom.lane@path.utah.edu.

This article contains supporting information online at www.pnas.orglookup/suppl/doi:10.1073/pnas.1406658111/-/DCSupplemental.
dorsal spinal cord during demyelinating disease progression (19–
23). However, because engrafted NPCs preferentially migrate to
regions deep within the ventral spinal cord (24), standard dorsal-
side 2P in vivo imaging techniques are not suitable for visuali-
zation deep in the ventral side. Using a ventral-side imaging
preparation, we now demonstrate that NPCs transplanted into
the spinal cords of JHMV-infected mice under pathologic con-
ditions migrate directionally, take up residence in regions of
axonal degradation, colocalize with damaged axons, and facil-
itate remyelination through direct interactions with axons.

Results
Live-Cell Imaging of Axonal Degeneration in the Ventral Spinal Cord.
To establish a system for ex vivo imaging in the ventral side of
the murine spinal cord, we isolated the spinal cord from thoracic
vertebra 4 to lumbar vertebra 2. The explanted spinal cord was
embedded in a 5% agarose gel to maintain spinal cord integrity
during superfusion with oxygenated medium (Fig. 1A) and was
subsequently imaged by 2P microscopy. We used transgenic Thy1-
YFP mice, which express yellow fluorescent protein (YFP) in
a subset of medium- to large-caliber axons (25, 26), to study ax-
onal pathology in the ventral spinal cord during the course of
demyelination following JHMV infection. Lesions in the ventral
spinal cord contained axons displaying a spectrum of “focal ax-
onal degeneration” (FAD) (an established scale of axonal dam-
age (20)) in morphologies (Fig. 1B). Long, continuous axons with no
damage are defined as “FAD stage 0”; FAD stage 1 axons con-
tain focal swellings progressing along the length of the axon; and
FAD stage 2 axons have gaps separating areas of YFP fluores-
cence. Analysis of FAD at various time points following JHMV
infection revealed a progression of axonal pathology (Fig. 1C),
similar to studies examining FAD in the dorsal spinal cord fol-
lowing myelin oligodendrocyte glycoprotein (MOG) immuniza-
tion (20). Whereas damaged axons were not observed in the
noninfected mouse (Fig. 1D), rapid FAD progression was readily
observed 7 d following JHMV infection, confirming that FAD
was a result of JHMV infection (Fig. 1E and Movie S1).

Whereas FAD has been shown to be associated with mito-
ochondrial and myelin damage (20), it is unknown whether FAD
stage 2 axons are completely transected. To determine the extent
of axonal transection in axons that had lost YFP signal, JHMV-
infected spinal cord sections were examined for axonal damage
by immunofluorescence microscopy of α-SMI-32 staining, which
detects a nonphosphorylated epitope in neurofilament H (27). In
areas where the axonal YFP signal was markedly diminished and
fractured, SMI-32 staining appeared punctate or absent, in-
dicating severely damaged axons (Fig. 2A). We observed various
stages of axonal damage along the length of a single axon (Fig.
2B), including areas of intact YFP signal without SMI-32 (intact
healthy axon), YFP signal concomitant with SMI-32 (intact but
damaged axon), no YFP or SMI-32 signal (transected area of
axon without continuous neurofilament), and SMI-32 without
YFP signal (intact but damaged axon). The latter case indicates
that although a loss of YFP fluorescence correlates with varying
degrees of axonal damage, it does not necessarily indicate complete
axonal transection. We conclude that loss of YFP signal correlates
with varying degrees of axonal damage, but not necessarily an
irreversibly transected axon. Whereas both FAD 1 and FAD 2 axons
had areas with axonal damage, axons exhibiting loss of both YFP
and SMI32 at multiple loci were more common in FAD 2 axons
(Fig. 2C). To verify that axonal regions devoid of both YFP and
SMI-32 signal are completely transected, we performed immuno-
histochemical staining for SMI-31, an accepted marker for un-
damaged axonal regions (phosphorylated neurofilament H) (28).
We were unable to detect SMI-31 without YFP signal along YFP-
expressing axons (Fig. 2D). These findings support the conclu-
sion that YFP SMI-32−axonal regions are transected.

Live-Cell Imaging of NPC Dynamics in the Ventral Spinal Cord.
To observe engrafted NPC behavior, GFP-NPCs were transplant-
ed intraspinally at thoracic vertebra 10 (5, 24, 29) and were
monitored ex vivo under 2P excitation. We first confirmed that
GFP-fluorescent cells were indeed NPCs and that the fluores-
cence did not arise from other cells that may have phagocytosed
GFP-NPCs. Spinal cord slices were stained for the ionized cal-
cium-binding adaptor molecule 1 (Iba-1), a marker of activated
macrophages and microglia (30). Despite a high number of ac-
tivated macrophages and microglia in the spinal cord 3 wk fol-
lowing transplant, GFP fluorescence did not overlap with Iba-1
staining (Fig. 3A), demonstrating that the GFP fluorescence
observed in the spinal cord was not due to engulfed NPCs and
this was consistent with earlier studies (5).

At day 1 following GFP-NPC transplantation into a non-
infected mouse, clusters of cells had limited motility at the site of
transplant (Fig. S1A). In control noninfected mice, GFP-NPCs
moved with an average velocity of 0.9 ± 0.1 μm·min⁻¹ (n = 5).
NPC motility characteristics were altered in the JHMV-infected
demyelinated spinal cord, where GFP-NPCs moved with a lower
average velocity of 0.7 ± 0.05 μm·min⁻¹ and a lower average
motility index (MI = 0.6 ± 0.1) compared to NPCs in the non-
infected spinal cord (29). (Movie S2). At day 1 posttransplant,
GFP-NPCs were observed distal to the transplant site in three of five noninfected and three of six JHMV-infected mice.
GFP-NPCs >300 μm from the transplant site had an av-
erage velocity of 0.5 ± 0.7 μm·min⁻¹ in the JHMV-infected spinal
cord, compared with 9.3 ± 0.6 μm·min⁻¹ in the noninfected
spinal cord (Fig. 3C). We applied directional persistence analysis
(31) to further characterize NPC migration to describe the di-
rection of cell movement over time; a value of 1 represents mi-
gration along a straight line without turning, 0 represents neutral
movement, and \( -1 \) represents migration directly toward the track origin (Methods). The directional persistence of transplanted NPCs was significantly lower in the JHMV-infected spinal cord, compared with the noninfected spinal cord (Fig. 3C).

When examined 14 d after transplant, GFP-NPCs were observed in dense clusters in the ventral area of the thoracic region of both the noninfected and the JHMV-infected spinal cord. A subset of GFP-NPCs expressed Ki-67, a nuclear marker of cellular proliferation (32), indicating proliferation occurred 2 wk after transplant (Fig. 4A). Proliferation was significantly increased within the JHMV-infected spinal cord, in which 55.7 \( \pm \) 6.8% of DAPI+ GFP-NPCs expressed Ki-67, compared with 14.4 \( \pm \) 2.2% in the noninfected spinal cord (Fig. 4B). Two-photon imaging revealed that GFP-NPCs exhibit dynamic morphologies in the ventral spinal cord. Cells were observed to proliferate, with large (\( \sim 10 \) μm) “buds” protruding off clusters of GFP-NPCs (Fig. 4C and Movie S3), and GFP-NPCs actively extended and retracted processes in both the noninfected and the JHMV-infected spinal cord (Fig. S2 and Movie S4).

**GFP-NPCs Preferentially Colocalize with Damaged Axons.** One week following NPC transplantation into the JHMV-infected Thy1-YFP mouse, GFP-NPCs closely associated with stage 1 and stage 2 FAD axons (Fig. 5A and B). Large clusters of GFP-NPCs preferentially established residence in regions with extensive axonal damage as determined by the FAD index (Fig. 5C). Following migration to FAD lesions, GFP-NPCs initiated intercellular interactions with stage 1 and stage 2 FAD axons (Fig. 5D and Movie S5), gathering YFP fluorescent segments of stage 2 axons together (Fig. 5E and Movie S6). Because loss of YFP signal does not necessarily indicate a transplanted axon, GFP-NPC contact with an axon may alter YFP fluorescence within intact FAD axons. Therefore, we examined GFP-NPCs that transiently contacted Thy1-YFP axons at 2 wk posttransplant. Axonal pathology has been associated with a decrease in YFP fluorescence (20), suggesting that NPC interactions with FAD axons may stabilize axons. In transient interactions, as the colocalization of an NPC and an axon diminished, YFP fluorescence rapidly decreased in the axon, changing the axon from FAD 1 to FAD 2 (Fig. 6A–C and Movie S7). Analysis of multiple GFP-NPCs revealed that the migration of NPCs away from damaged axons was strongly correlated with the loss of axonal YFP fluorescence (Fig. 6D).

**Engrafted GFP-NPCs Remyelinate Axons.** To determine whether transplanted NPCs produce myelin following interactions with damaged axons, we cultured NPC neurospheres isolated from mice that express GFP under the control of the myelin proteolipid protein promoter (PLP-GFP) (33). Two weeks following transfer into a JHMV-infected spinal cord, NPCs formed stable interactions with damaged axons and expressed GFP, indicating that transplanted NPCs differentiated into myelinating cells and expressed myelin genes (Fig. 7A). In these cells, GFP fills the cytoplasm of the cell body, revealing that differentiated NPCs wrapped around damaged axons (Movie S8), with the GFP fluorescence extending around the length of the axon (Fig. 7B). Correspondingly, YFP fluorescence increased (Fig. 7C), indicating increased axonal health based on earlier reports correlating axonal health/integrity with YFP expression (20). Furthermore, we found GFP-NPCs wrapped around YFP+ axons and myelin basic protein (MBP)
colocalized with GFP fluorescence, further confirming that transplanted NPCs actively participate in remyelination (Fig. 7D and Fig. S3).

Discussion

Our results indicate that transplanted GFP-NPCs preferentially migrate to regions of axonal damage, proliferate, and actively remyelinate axons. NPCs have been shown to selectively colonize areas of white matter damage and facilitate remyelination in preclinical animal models of neuroinflammatory demyelination (5, 8, 34, 35). However, the migration of transplanted NPCs and their interactions with damaged axons has not previously been visualized. In this study, we established a system for stable ex vivo imaging to observe single NPC behavior in the ventral spinal cords of mice, using a model of viral-induced demyelination. Although the etiology of MS is unknown, numerous factors including both genetic and environmental influences are considered important in initiation and maintenance of disease. Viral infection has long been considered a potential triggering mechanism involved in demyelination, and numerous human viral pathogens have been suggested to be involved in eliciting myelin-reactive lymphocytes and/or antibodies that subsequently infiltrate the CNS and damage the myelin sheath (36–39). As such, viral models of neuroinflammation/demyelination are relevant and have provided important insights into mechanisms associated with disease. Moreover, the molecular mechanisms governing how engrafted NPCs interact with demyelinated axons within an inflammatory environment resulting from demyelination derived from a persistent viral infection have not been defined. Therefore, the present study uses the JHMV model of demyelination to characterize NPC migration kinetics as well as their ability to physically engage demyelinated axons and promote remyelination. To accomplish this, we used transgenic Thy1-YFP mice and 2P microscopy to visualize axonal damage following JHMV infection and imaged real-time interactions of transplanted GFP-NPCs with damaged axons, allowing insight into mechanisms by which transplanted NPCs contribute to amelioration of clinical and histopathological disease.

Axonal damage in MS is considered a secondary event that occurs following myelin loss in response to accumulation of myelin-reactive lymphocytes within the CNS and this is supported by experimental autoimmune encephalomyelitis (EAE), an autoimmune model of neuroinflammation and demyelination. More recently, the use of viral models of demyelination as well as EAE, emerging evidence supports the possibility that axonal damage precedes demyelination (40–44). Within 1 wk following infection of Thy1-YFP mice with JHMV, we observed axons with FAD, characterized by discontinuous YFP fluorescence, consistent with earlier reports describing axonopathy in JHMV-infected mice (41, 45). Our findings indicate that axonal damage occurs early following JHMV infection of the CNS before robust immune-mediated demyelination. Previous in vivo imaging studies of the demyelinated spinal cord suggest that loss of fluorescence in axons indicates total axonal transsection (20, 23). However, we show that loss of YFP fluorescence can occur in an intact axon and does not necessarily mean axonal transection. Although it is possible a variety of cell types may interact with axonal segments, we show that axonal YFP fluorescence changes reversibly during and subsequent to interaction with transplanted NPCs, judged by colocalization with transplanted NPCs. The observed variability in the amount of axonal YFP fluorescence change may be a result of the extent of axonal damage at the time of interaction or the length of the imaging window. These results extend previous studies from our laboratory that demonstrated axonal sparing following NPC transplantation into JHMV-infected mice (8).

Physical engagement of damaged axons by transplanted NPCs increases axonal YFP fluorescence, and disengagement of NPCs

Fig. 4. GFP-NPCs proliferate extensively in the JHMV-infected spinal cord. (A) Immunostaining in transverse sections of JHMV-infected spinal cords 2 wk after GFP-NPC transplantation. Ki-67 (red), GFP-NPCs (green), and colocalization between overlapping GFP-NPC and Ki-67 fluorescence were determined using the Imaris colocalization tool (white). (Right) Merge of all three channels. (B) Quantification of the number of Ki-67+/GFP+ nuclei divided by the number of DAPI+/GFP+ nuclei in multiple spinal cord sections from transplanted mice. (C) Time-lapse images of a GFP-NPC cluster expanding at the indicated time points (min:s) in a noninfected spinal cord 7 d posttransfer (Movie S3). (Scale bars, 10 μm.)

Fig. 5. GFP-NPCs colocalize and initiate intercellular interactions with damaged axons. (A) Representative image showing colocalization between GFP-NPCs (teal) and stage 1 FAD+ axons (yellow) in the JHMV-infected Thy1-YFP spinal cord 8 d posttransfer. Endogenous spinal cord structures (collagen) are visualized by second harmonic generation (blue). (B) Representative image from the same spinal cord as in A, showing increased colocalization between GFP-NPCs and stage 2 FAD+ axons in a more damaged lesion in the JHMV-infected Thy1-YFP spinal cord 8 d posttransfer. (C) GFP-NPC localization correlates with the FAD severity of lesions in the JHMV-infected Thy1-YFP spinal cord 8 d posttransfer. Number of transferred GFP-NPCs found in lesions is plotted vs. FAD severity of the lesions for each mouse with the polynomial fit in red. Multiple lesions in three separate mice are shown, with different symbols (♦, ●, ○) representing each mouse with the polynomial fit in red. Correlation coefficient (r): 0.78. (D) Time-lapse images showing GFP-NPCs initiating intercellular interactions with stage 1 FAD axons in the JHMV-infected Thy1-YFP spinal cord 8 d posttransfer. Circle indicates a GFP-NPC actively participating in remyelination (Fig. 7D and Fig. S3). (Scale bars, 10 μm.)
from axons rapidly diminishes YFP signal intensity. Loss of YFP fluorescence may occur in intact axons due to impaired microtubular transport (46), axonal swelling resulting in retraction of proteins from damaged areas (47), changes in reactive oxygen and nitrogen species that initiate mitochondrial pathology (48), or changes in ion channel expression (49) that do not allow for a favorable environment for YFP fluorescence. However, to account for the rapid changes in YFP fluorescence, we propose that interactions with NPCs may reverse acidification and diminish elevated internal calcium concentrations present in axons in inflammatory lesions through effects on NMDA receptors (23) or acid-sensing ion channels (50). YFP fluorescence is particularly sensitive to acidic pH (51), indicating that restoration of normal physiological pH may underlie rapid increases in axonal YFP fluorescence observed during remyelination. Continued studies examining these possibilities are required to better understand the nature of axonal damage in response to CNS viral infection and the ability of NPCs to protect and repair damaged axons.

We have previously shown that transplanted GFP-NPCs preferentially colonize areas of demyelination and this positional migration is guided, in part, by expression of the chemokine receptor CXCR4 on transplanted NPCs that respond to enriched expression of the chemokine ligand CXCL12 that is enriched within areas of white matter pathology (5). However, the motility of engrafted NPCs was not examined. Our results support and extend those earlier findings by showing that NPCs recognize and directly interact with damaged axons. Further, we now demonstrate motility differences between NPCs transplanted into a demyelinated spinal cord and NPCs transplanted into a non-infected spinal cord. One day following transplant into the JHMV-infected spinal cord, the motility of GFP-NPCs was slower than in the absence of infection. This result may be due to GFP-NPCs initiating contact with demyelinated axons. In support of this, we demonstrate that transplanted GFP-NPCs extended processes and make extensive contacts with damaged axons. However, it is possible that reduced GFP-NPC motility is due to inflammatory signals in the JHMV-infected spinal cord. The molecular signals mediating this interaction are to this point undefined, although previous studies have clearly shown that NPCs are capable of expressing adhesion molecules that may engage axons (52).

Engagement of damaged axons by transplanted GFP-NPCs is the first step in events leading to NPC differentiation and remyelination (53, 54). We have previously shown that the majority of transplanted NPCs into JHMV-infected mice differentiate into oligodendroglia, the myelinating cells of the CNS supporting an important role for transplanted cells in participating in remyelination (5, 8). Moreover, transplantation of NPCs lacking the transcription factor Olig1 into JHMV-infected mice results in diminished clinical and histologic recovery that correlates with altered lineage fate commitment as Olig1-deficient NPCs preferentially differentiate into astrocytes (55). Until now, studies have shown that transplanted NPCs directly or indirectly contribute to endogenous remyelination (11, 55), although evidence of direct remyelination...
was not entirely conclusive. Whereas we have previously shown that GFP expression from transplanted NPCs colocalized with MBP (55), our real-time imaging of PLP-expressing NPCs definitively demonstrates that transplanted NPCs directly remyelinate axons and are not merely stimulating endogenous oligodendroglia to remyelinate. We show that NPC-derived oligodendrocytes express PLP, a protein necessary for myelination, and extend and cover axons within ~20 min. Finally, this study also provides a model system to better understand the physical nature of NPC interactions with damaged axons.

Methods

Mice and Virus. C57BL/6 (National Cancer Institute) and Thy1-YFP (25) (Jackson Laboratory) mice were isolated intracranially (i.c.) with 150 plaque-forming units (PFU) of JHMV strain J2.2v-1 (JHMV) in 30 μl sterile Hank’s balanced saline solution (HBSS) (5, 24). Mice were killed by inhalation of halothane (Sigma-Aldrich) at various days postinfection (p.i.), and spinal cords were removed and processed for analysis. For tissues used for immunohistochemistry, mice were fixed by cardiac perfusion. Mice expressing eGFP driven by the mouse myelin PLP gene promoter were used to generate NPCs that express eGFP only, following terminal differentiation into mature myelinating oligodendrocytes (33). All experiments were approved by the University of California, Irvine, Institutional Animal Care and Use Committee.

Cell Culture, Reagents, and Transplantation. Enhanced green fluorescent protein-expressing NPCs (GFP-NPCs), derived from C57BL/6 mice, were cultured as previously described (24). In addition, NPCs from PLP-GFP mice were isolated and cultured before transplantation as previously described (34, 55). Following culture in DMEM:F12 (Invitrogen) supplemented with 10% FCS and 1% penicillin/streptomycin, 10 ng/mL T3 (Sigma-T67407), and 20 ng/mL human recombinant EGF (Sigma-Aldrich), PLP-GFP neurospheres were dissociated with 1× TrypLE (Gibco-12563-011) for 20 min at 37 °C and centrifuged for 5 min at 500 × g.

For optimal cutting temperature compound and resin-embedded sections as described for immunohistochemistry were used. DAPI Fluoromount-G (Southern Biotech) was used to visualize nuclei. Colocalization of overlapping fluorescence was determined using Imaris 7.3 (Bitplane). Pixel intensities over a set threshold at the same position were considered colocalized.

Spiral Cord Preparation, Two-Photon Imaging, and Analysis. Two-photon imaging was performed using a previously described system (56) with a Chameleon Ultra II Ti:Sapphire laser (Coherent). Spinal cords were isolated from thoracic vertebra 4 to lumbar vertebra 2, embedded in 5% agarose gel to maintain the integrity of the spinal cord, oriented with the ventral side facing the dorsal surface, and cultured before transplantation as previously described (55). Following culture in DMEM:F12 (Invitrogen) supplemented with 10% FCS and 1% penicillin/streptomycin, 10 ng/mL T3 (Sigma-T67407), and 20 ng/mL human recombinant EGF (Sigma-Aldrich), PLP-GFP neurospheres were dissociated with 1× TrypLE (Gibco-12563-011) for 20 min at 37 °C and centrifuged for 5 min at 500 × g. Axonal degradation index was calculated based on an axonal degradation index previously described (20):

\[
\text{FAD index} = \frac{\text{# of stage 1 axons} \times 1 + \# \text{ of stage 2 axons} \times 2}{\# \text{ axons}}
\]

Immunohistochemistry. The murine spinal cord was extracted and processed for optimal cutting temperature compound and resin-embedded sections as previously described (8). For immunofluorescence staining, slides were prepared with goat serum and block BSA as goat serum in 1× PBS + 0.5% BSA from goat serum previously described (8). Primary antibodies included rabbit anti-ib1 (1 mg/ml, Wako Chemicals), rabbit anti-MBP (1:200; Chemicon), mouse anti-SMI-31 (phosphorylated neurofilament H; 1:1,000; EMD Millipore), or mouse anti-SMI-32 (nonphosphorylated neurofilament H; 1:1,000; Covance). Anti-SMI-31 and anti-SMI-32 were blocked as described above with the addition of 5% (vol/vol) purified goat anti-mouse IgG (Invitrogen). The secondary antibody used for anti-ib1 and anti-MBP was Alexa 594 goat anti-rabbit (1:1,000; Invitrogen); for anti-SMI-31 and anti-SMI-32, Alexa 594 goat anti-mouse (1:1,000; Invitrogen) was used. DAPI Fluoromount-G (Southern Biotech) was used to visualize nuclei. Colocalization of overlapping fluorescence was determined using 7.3 (Bitplane). Pixel intensities over a set threshold at the same position were considered colocalized.

Statistical Analysis. Statistical significance for velocity measurements in Fig. 3C was determined using Student’s t test. The Mann–Whitney U test was used to calculate significance for the nonnormally distributed directional persistence measurements in Fig. 3D. A P value <0.05 was considered significant. Data are presented as mean ± SEM.

ACKNOWLEDGMENTS. We acknowledge the California Institute for Regenerative Medicine core facilities at the University of California, Irvine. This work was supported in part by National Institutes of Health (NIH) Grants R01 GM-41514 (to M.D.C.) and R01 NS-074987 (to T.E.L.) and the National Multiple Sclerosis Society (NMSS) Collaborative Center Research Award CA1058-A-8 (to T.E.L. and M.D.C.), NMSS Grant RG4925, NIH Training Grant T32 NS070373 (to M.L.G.), and NMSS Postdoctoral Fellowship FG 1960-A-1 to J.G.W.

REFERENCES


where D equals displacement and d equals distance, which was calculated for every two steps for sequential 3D coordinates of cell position (31). FAD index of MS-like lesions was calculated based on an axonal degradation index previously described (20):


Fig. S1. GFP-neural precursor cell (GFP-NPC) dynamics are limited at the injection site. (A) Snapshot of GFP-NPCs (green) at the injection site 1 d following transfer into the JHM strain of mouse hepatitis virus (JHMV)-infected spinal cord. (B) Distribution of instantaneous GFP-NPC cellular velocities at the injection site. Overall mean ± SEM is indicated (>300 time points; $P < 0.0001$). (Scale bar, 10 μm.)

Fig. S2. GFP-NPCs actively extend processes after engraftment. (A) Time-lapse images of a GFP-NPC actively extending and retracting processes at the indicated time points in a noninfected spinal cord 7 d posttransfer. (min:s) (B) Time-lapse images showing GFP-NPCs actively extending processes in the JHMV-infected C57BL/6 spinal cord 14 d posttransfer. Panels depict dynamic behavior of individual GFP-NPCs over the timescale indicated (Movie S4). (Scale bars, 10 μm.)

Fig. S3. Immunostaining in transverse sections of JHMV-infected Thy1-YFP spinal cords after GFP-NPC transplantation. Myelin basic protein (MBP) (red), yellow fluorescent protein (YFP)+ axons (yellow), and colocalization between overlapping GFP-NPC (green) and MBP fluorescence were determined using the Imaris colocalization tool (white). (Right) Merge of all three channels. (Scale bar, 4 μm.)
Movie S1. Focal axonal degeneration (FAD) progression in the ventral spinal cord. Thy1-YFP\textsuperscript{+} axons (yellow) are visualized under two-photon (2P) excitation in the explanted ventral spinal cord 1 wk following JHMV infection. Axons do not exhibit FAD morphology in the noninfected spinal cord (Left). FAD is observed in the JHMV-infected spinal cord (Right) with areas of active pathology highlighted in red. Movie duration = 31:06 (min:s). (Scale bar, 20 μm.)

Movie S2. GFP-NPC motility in the ventral spinal cord. GFP-NPCs (green) are visualized under 2P excitation in regions >300 μm from the transplant site 1 d following transplant into C57/Bl6 mice. Movie duration = 14:24 (min:s). (Scale bar, 20 μm.)

Movie S3. Dense clusters of GFP-NPCs in the ventral spinal cord. GFP-NPCs (green) are visualized under 2P excitation 1 wk following transplant into a C57/Bl6 mouse. Shown is a 3D rotation of the GFP-NPC cluster, followed by a time-lapse movie of GFP-NPC proliferation. Movie duration = 25:05 (min:s). (Scale bar, 40–10 μm.)
Movie S4. GFP-NPCs extend processes in the ventral spinal cord. GFP-NPCs (green) are visualized under 2P excitation 1 wk following transplant into a C57/Bl6 mouse. Dynamic GFP-NPC processes are highlighted by white arrows. Movie duration = 7:13 (min:s). (Scale bar, 10 μm.)

Movie S5. NPC–axon interactions in the ventral spinal cord. GFP-NPCs (teal) and Thy1-YFP⁺ axons (yellow) are visualized under 2P excitation 1 wk following transplant into a JHMV-infected Thy1-YFP spinal cord. Interactions between the NPC and the axon are highlighted in red. Collagen (blue) is visualized by second-harmonic generation. Movie duration = 6:32 (min:s). (Scale bar, 10 μm.)
Movie S6. NPCs manipulate YFP+ axonal segments in the ventral spinal cord. GFP-NPCs (teal) and Thy1-YFP+ axons (yellow) are visualized under 2P excitation 1 wk following transplant into a JHMV-infected Thy1-YFP spinal cord. Movie duration = 18:53 (min:s). (Scale bar, 10 μm.)
Movie S8. NPCs express myelin proteolipid protein (PLP) and wrap around damaged axons. PLP-GFP-NPCs (green) and Thy1-YFP+ axons (yellow) are visualized under 2P excitation 2 wk following transplant into a JHMV-infected Thy1-YFP spinal cord. PLP-GFP wrapping is highlighted in red. Movie duration = 36:11 (min:s). (Scale bar, 10 μm.)

Movie S7.

Axonal YFP fluorescence decreases as NPCs dissociate from axons. GFP-NPCs (teal) and Thy1-YFP+ axons (yellow) are visualized under 2P excitation 2 wk following transplant into a JHMV-infected Thy1-YFP spinal cord. Movie duration = 104:21 (min:s). (Scale bar, 10 μm.)

Movie S7