● PERSPECTIVE

Use of longitudinal magnetic resonance imaging in preclinical models of spinal cord injury

Spinal cord injury (SCI) is a distressing event with grave socio/economic consequences to our society. Pathophysiological response following SCI involves blood-spinal cord barrier breakdown, neuroinflammation and formation of a glial scar that altogether govern the feasibility of spontaneous axonal re-growth and limited functional recovery. Great advances in understanding SCI pathophysiology have been achieved using numerous transgenic mouse lines developed in different strains. However, there are inherent strain differences that affect inflammation, gliosis, axon regeneration and ultimately functional recovery after SCI.

Mouse strain has a profound effect on functional recovery after SCI: Initially, Ma and co-workers (2004) found increased inflammation and reduced axon re-growth in C57BL/6 compared to 129X1-SvJ mice following moderate spinal cord contusion (Ma et al., 2004). Subsequently, Kigerl and colleagues (2006) also showed increased inflammation in C57BL/6 compared to C57BL/10, BALB/c and B10.PL mouse strains after moderate spinal cord contusion (Kigerl et al., 2006). Likewise, following severe spinal cord contusion, C57BL/6 displayed elevated inflammation compared to BALB/c mice (Kerr and David, 2007).

Interestingly, C57BL/6 shows reduced astrocyte reactivity than 129X1-SvJ mice after moderate contusion (Ma et al., 2004) and lateral spinal cord hemisection (Dixon et al., 2012). Similarly, we recently found reduced gliosis in C57BL/6 compared to Swiss Webster mice following both lateral hemisection (Noristani et al., 2018b) and transection of the spinal cord (Noristani et al., 2018a).

C57BL/6 mice display better spontaneous functional recovery compared to Swiss Webster background after lateral hemisection of the spinal cord (Noristani et al., 2018b). However, following transection of the spinal cord, C57BL/6 mice show reduced mobility than Swiss Webster mice (Noristani et al., 2018a).

Altogether, these data demonstrate strain-dependent differences in pathophysiological response after SCI. This affects functional recovery in mice and needs to be considered in pre-clinical findings.

MRI analysis in preclinical models of SCI: In the clinics, non-invasive examination of the lesion evolution is only possible using magnetic resonance imaging (MRI).

In vivo MRI in mice is particularly challenging because of the small physical size of the spinal cord. Previous *in vivo* MRI studies in mice predominantly focused on C57BL/6 mice at multiple time-points after SCI including 3 hours (Kim et al., 2010), 24 hours (Bonny et al., 2004), 2 weeks (Kim et al., 2007) and up to 4 weeks (Bilgen et al., 2007). We have also recently shown that T2-weighted *in vivo* ^{1}H-MRI using a 9.4 Tesla apparatus accurately assesses lesion-induced tissue alterations. In particular, we demonstrated that *in vivo* ^{1}H-MRI findings closely correlates with high resolution T2-weighted *ex vivo* ^{1}H-MRI and general histology after several SCI in both Swiss Webster mice (Noristani et al., 2015) and in non-human primate (Le Corre et al., 2018). Until recently it was unclear whether either *in vivo* or *ex vivo* ^{1}H-MRI analyses could detect slight differences in lesion evolution such as strain-dependent variances in neuroinflammation and gliosis following SCI.

To address this issue, we carried out detailed behavioral, T2-weighted *in vivo* and *ex vivo* ^{1}H-MRI as well as histological assessments in mice with C57BL/6 and Swiss Webster backgrounds (Noristani et al., 2018b). We chose lateral hemisection of the spinal cord at thoracic level 9 vertebra that allowed examination of spontaneous functional recovery

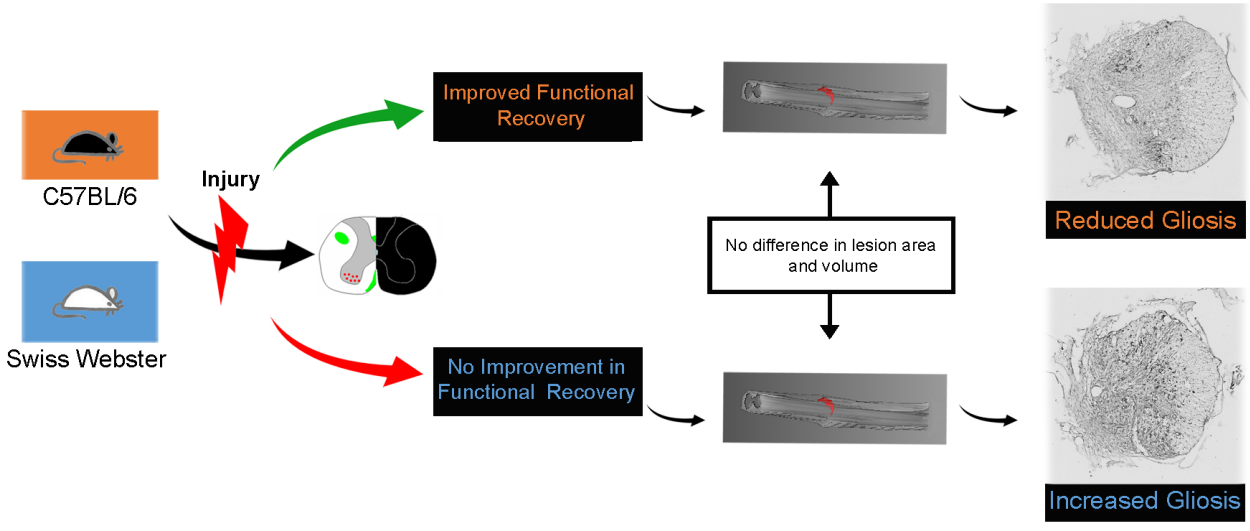

Figure 1 Longitudinal behavior, MRI and histological examination of the spinal cord in two commonly used mouse strains.
The two mouse strains underwent lateral hemisection of the spinal cord followed by CatWalk™ examination of spontaneous functional recover, T2-weighted ^{1}H-MRI analysis and immunohistochemical assessment of gliosis. C57BL/6 mice displayed better spontaneous functional recovery associated with reduced gliosis compared to mice with Swiss Webster background. T2-weighted *in vivo* ^{1}H-MRI showed similar changes in lesion expansion and volume; findings that were subsequently confirmed by high resolution T2-weighted *ex vivo* ^{1}H-MRI and classical histology.

(**Figure 1**). We found that mice with C57BL/6 background had better inter-paw coordination recovery and improved hind paw weight support compared to Swiss Webster mice. In addition, C57BL/6 mice displayed improved bodyweight recovery and reduced post-injury anxiety than Swiss Webster mice. Using T2-weighted *in vivo* 9.4 Tesla MRI we clearly identified the lesion site. We observed the highest increase in lesion volume within a week post-lesion in both C57BL/6 and Swiss Webster backgrounds. This most likely reflects the early vasogenic edema and plasma leakage in the lesion core resulting from blood-spinal cord barrier damage (Noristani et al., 2015). T2-weighted *in vivo* ^{1}H-MRI found no strain-dependent differences in lesion volume over 3 months after SCI (Noristani et al., 2018b). These findings were subsequently confirmed using high resolution T2-weighted *ex vivo* ^{1}H-MRI and classical histology. However, using immunohistochemistry, we observed reduced gliosis in C57BL/6 compared to Swiss Webster mice (Noristani et al., 2018b). C57BL/6 mice displayed reduced microglia/macrophage reactivity not only within the epicenter but also rostro-caudal to the lesion than Swiss Webster mice. Furthermore, C57BL/6 had increased serotonergic axon density after SCI than Swiss Webster mice.

Perspective: Our recent data highlight that T2-weighted *in vivo* ^{1}H-MRI reliably quantifies lesion expansion and volume. This may be an alternative to classical histology in pre-clinical models of SCI. However, future advances in MRI field are necessary to achieve in-depth analysis of tissue reorganization, plasticity and adaptation of the remaining circuitries following SCI. Conventional T2-weighted ^{1}H-MRI cannot discriminate fibrous structures including the white matter tract and the structural integrity of axon bundles that are critical in SCI context. On the other hand, diffusion weighted MRI (dw-MRI) reflects local water molecule dynamics that can be used to better characterize tissue structure. Dw-MRI uses water molecule diffusion along a favored direction to assess tissue integrity and structure. In the spinal cord, water molecule diffusion along the rostro-caudal direction reflects axonal integrity in the white matter and is usually decreased after lesion. *Ex vivo* dw-MRI also has higher signal-to-noise-ratio compared to *ex vivo* T2-weighted images. This allows better discrimination between the injured and intact tissue. In SCI context, dw-MRI is particularly useful to evaluate myelin integrity and glial cell reactivity. Indeed, using high resolution *ex vivo* dw-MRI, we recently identified a marked increase in microglial/monocytes density early after SCI (Noristani et al., 2017). Future studies using dw-MRI may allow more in-depth analyses of lesion evolution including inflammation, gliosis and axonal integrity in pre-clinical models of SCI.

Harun Najib Noristani[*], **Florence Evelyne Perrin**[*]
INSERM U1198, University of Montpellier, Montpellier, France
***Correspondence to:** *Harun Najib Noristani, harun.noristani@ibn-lab.com; Florence Evelyne Perrin, PhD, florence.perrin@inserm.fr.*
***orcid:** 0000-0001-7742-140X (Harun Najib Noristani)*
0000-0002-7630-0515 (Florence Evelyne Perrin)
***Received:** October 4, 2018*
***Accepted:** December 5, 2018*
***doi:** 10.4103/1673-5374.249222*

***Plagiarism check:** Checked twice by iThenticate.*
***Peer review:** Externally peer reviewed.*

***Open peer reviewers:** Alessandra Bitto, University of Messina, Italy; Hailong Song, University of Missouri Columbia, USA.*
***Additional file:** Open peer review reports 1 and 2.*

References

Bilgen M, Al-Hafez B, Alrefae T, He YY, Smirnova IV, Aldur MM, Festoff BW (2007) Longitudinal magnetic resonance imaging of spinal cord injury in mouse: changes in signal patterns associated with the inflammatory response. Magn Reson Imaging 25:657-664.

Bonny JM, Gaviria M, Donnat JP, Jean B, Privat A, Renou JP (2004) Nuclear magnetic resonance microimaging of mouse spinal cord in vivo. Neurobiol Dis 15:474-482.

Dixon KJ, Munro KM, Boyd AW, Bartlett PF, Turnley AM (2012) Partial change in EphA4 knockout mouse phenotype: loss of diminished GFAP upregulation following spinal cord injury. Neurosci Lett 525:66-71.

Kerr BJ, David S (2007) Pain behaviors after spinal cord contusion injury in two commonly used mouse strains. Exp Neurol 206:240-247.

Kigerl KA, McGaughy VM, Popovich PG (2006) Comparative analysis of lesion development and intraspinal inflammation in four strains of mice following spinal contusion injury. J Comp Neurol 494:578-594.

Kim JH, Loy DN, Liang HF, Trinkaus K, Schmidt RE, Song SK (2007) Noninvasive diffusion tensor imaging of evolving white matter pathology in a mouse model of acute spinal cord injury. Magn Reson Med 58:253-260.

Kim JH, Loy DN, Wang Q, Budde MD, Schmidt RE, Trinkaus K, Song SK (2010) Diffusion tensor imaging at 3 hours after traumatic spinal cord injury predicts long-term locomotor recovery. J Neurotrauma 27:587-598.

Le Corre M, Noristani HN, Mestre-Frances N, Saint-Martin GP, Coillot C, Goze-Bac C, Lonjon N, Perrin FE (2018) A novel translational model of spinal cord injury in nonhuman primate. Neurotherapeutics 15:751-769.

Ma M, Wei P, Wei T, Ransohoff RM, Jakeman LB (2004) Enhanced axonal growth into a spinal cord contusion injury site in a strain of mouse (129X1/SvJ) with a diminished inflammatory response. J Comp Neurol 474:469-486.

Noristani HN, They L, Perrin FE (2018a) C57BL/6 and Swiss Webster mice display differences in mobility, gliosis, microcavity formation and lesion volume after severe spinal cord injury. Front Cell Neurosci 12:173.

Noristani HN, Saint-Martin GP, Cardoso M, Sidiboulenouar R, Catteau M, Coillot C, Goze-Bac C, Perrin FE (2018b) Longitudinal MRI analysis and histological characterization after spinal cord injury in two mouse strains with different functional recovery: gliosis as a key factor. J Neurotrauma doi: 10.1089/neu.2017.5613

Noristani HN, Lonjon N, Cardoso M, Le Corre M, Chan-Seng E, Captier G, Privat A, Coillot C, Goze-Bac C, Perrin FE (2015) Correlation of in vivo and ex vivo (1)H-MRI with histology in two severities of mouse spinal cord injury. Front Neuroanat 9:24.

Noristani HN, Boukhaddaoui H, Saint-Martin G, Auzer P, Sidiboulenouar R, Lonjon N, Alibert E, Tricaud N, Goze-Bac C, Coillot C, Perrin FE (2017) A combination of ex vivo diffusion MRI and multiphoton to study microglia/monocytes alterations after spinal cord injury. Front Aging Neurosci 9:230.

P-Reviewers: Bitto A, Song H; C-Editors: Zhao M, Wang JJ; T-Editor: Liu XL