Microcebus murinus: A novel promising non-human primate model of spinal cord injury

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● PERSPECTIVE

Microcebus murinus: a novel promising non-human primate model of spinal cord injury

The number of people affected by spinal cord injuries (SCI) ranges from 2.5 to 4 million worldwide. Traumatic SCI induces a primary injury due to initial mechanical impact that causes focal cellular and blood-spinal cord barrier damages. Subsequently, secondary injuries resulting from infiltration of peripheral monocytes, ischemia, edema, inflammation, glial scar and cystic cavities formation as well as excitotoxicity trigger cellular demise and prevent spontaneous axonal regrowth. Secondary damages, including aggregation of extracellular matrix protein at the lesion epicenter, thus greatly amplify impairments induced by the initial mechanical damage.

Spinal cord injury across species: Many differences in neuroanatomical organization of the motor and sensory systems between rodents and primates may partially explain why responses to SCI vary considerably between species (Courtine et al., 2007). In particular, the corticospinal tract architecture in primates could explain their superior functional recovery after lateralized injuries compared to rodents (Friedli et al., 2015). The glial scar, resulting from SCI, is composed of activated astrocytes and microglia and forms a barrier to spontaneous axon regeneration. Temporal differences in glial response had been also reported between rodents and primates (Miller et al., 2012). In particular, in *Macaca fascicularis*, there is no substantial astrogliosis surrounding the lesion site (Wu et al., 2013). There is, however, an evident microglia/macrophage activation not only at the lesion site but also in adjacent spared tissues. Gene expression analysis in marmosets revealed a delayed glial scar formation and a prolonged inflammatory response compared to rodents, further supporting temporal differences in glial response after injury among species (Nishimura et al., 2014). Several non-human primate models have been developed to study the underlying mechanisms involved in SCI. Nevertheless, some limitations remain associated with these available non-human primate models of SCI. *Macaca* not only present a rather large body size that makes their handling more challenging but they also display a delayed sexual maturity, a low reproductive output and a long inter-birth interval (Fischer and Austad, 2011). Thus, smaller non-human primate model such as *Callithrix Jacchus* (marmosets) is also used in the SCI field (Okano et al., 2012). Iwanami and collaborators (Iwanami et al., 2005) have developed a model of graded contusive SCI at cervical level 5 in common marmoset. They assessed the motor function using grasping and cage-climbing tests as well as measurements of spontaneous motor activity. Magnetic resonance imaging follow up was done using a 1.5-Tesla apparatus and histological analyses were performed. However, no precise evaluation of glial cells response was reported.

Another small non-human primate, the grey mouse lemur (*Microcebus murinus*), presents many physiological and neuroanatomical similarities with human. In addition, their small size, rapid sexual maturity, high reproductive output and a short inter-birth interval makes them particularly useful for SCI investigations. However, the anatomical characterization of its spinal cord was rather limited. We thus first established a mini atlas to document the anatomical organization of the rachis and the spinal cord of *Microcebus murinus* using computerized tomography, *ex vivo* diffusion magnetic resonance imaging (MRI) and histological staining (Le Corre et al., 2017).

Microcebus murinus as a novel model of spinal cord injury

***Injury model*:** We then developed a new model of lateral spinal cord hemisection in *Microcebus murinus* (**Figure 1A**) and carried out detailed behavioral assessments (Le Corre et al., 2017). Following laminectomy of the posterior arch of the first lumbar vertebra, a lateral hemisection of the spinal cord was done under microscope using a micro-knife (**Figure 1B**). We also used longitudinal T2-weighted

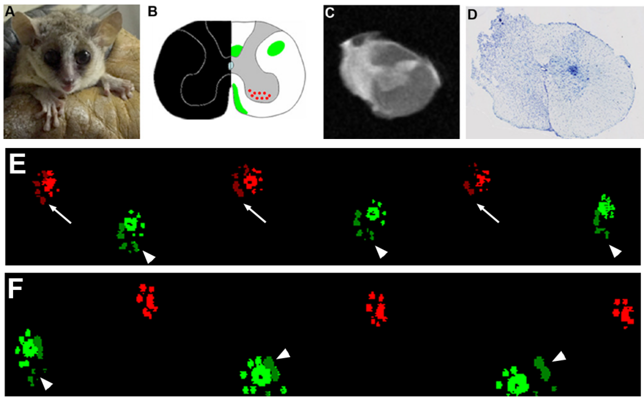

Figure 1 Characterization of a new non-human primate model of spinal cord injury (SCI).
(A) *Microcebus murinus* (3 years of age, 80 g). (B) Schematic view of a lateral hemisection of the spinal cord at lumbar level 1. The black area represents the lesioned side of the spinal cord. (C) *Ex vivo* magnetic resonance imaging (MRI) at the spinal cord lesion site 3 months after lateral hemisection. (D) Toluidine blue staining at the injured site 3 months after SCI. (E) CatWalk patterns of *Microcebus murinus* before injury. Green: Right paws; red: left paws. Mat (arrows and arrow heads): hind paws; bright: front paws. (F) The left hind paw print (red mat) is not present 3 days following injury.

in vivo MRI to follow lesion evolution up to 3 months post-injury, followed by post-mortem *ex-vivo* high resolution T2-weighted-MRI (**Figure 1C**) and detailed histological assessments (**Figure 1D**).

Behavioral and imaging assessments: To evaluate post-operative deficits, characterized by hind limb monoplegia ipsilateral to the lesion; we developed three different behavioral tests that were all performed 10, 5, and 2 days prior to surgery and then at 1, 3 and 7 days post-surgery. First, for open field test that consists of an evaluation of the spontaneous functional motor activity, lemurs were placed in an empty test arena and observed by two independent experimenters for at least 10 minutes and scored from 0 (no movement) to 6 (normal walking) . The open field test revealed severe motor deficits of the hind limb ipsilateral to the lesion up to 6 days post-lesion followed by a progressive recovery reaching over 80% of their initial scoring from 14 days onwards. The contralateral limb showed a transient impairment up to day 30 post-injury and then returned to normal. To evaluate fine motor function, we developed a grip test that consists of scoring the ability to grip a bar from 0 (no attempt to grip) to 2 (normal grip). This test allowed identifying a gradual recovery of the hind limb ipsilateral to the lesion up to 70 days post-injury followed by a plateau at 75% of their initial scoring. The difference between the two hind limbs persisted up to the end of the experiment at 90 days post-injury. In parallel, we also performed automated gait analysis using CatWalk (Noldus, Wageningen, Netherlands) with many parameters analyzed related either to individual footprints (such as width and length of a complete paw print, pressure exerted by a paw, duration of contact of a paw with the glass plate, area of the print at max contact) or to the position of footprints in a step cycle (stride length, base of support, relative print position). CatWalk showed significant alterations in motor function of the hind limb ipsilateral to the lesion up to 6 days after surgery, followed by a recovery period and a final return to normal from 14 days post-injury (**Figure 1E, F**).

In-vivo T2-weighted MRI (48 hours, 1, 4 and 12 weeks post-lesion) permitted to identify tissue re-organization at the lesion site over time resulting in an initially hypo-intense signal that was ultimately followed by a hyper-intense signal). Subsequent *ex vivo* T2-weighted MRI (**Figure 1C**) and histological assessments (**Figure 1D**) permitted to quantify the percentage of damage tissue at the epicenter. No difference between the three modalities (*in* & *ex vivo* MRI and histology) were observed with approximately 40% of damage area at the epicenter at 3 months post-injury.

Histological assessments: We next examine injury induced glial reactivity. We demonstrated a pronounced increase in glial fibrillary acidic protein (GFAP, 1:500; Dako, Glostrup, Denmark) expression at the lesion epicenter as well as adjacent to the lesion site (restricted to the grey matter rostral to the lesion). We also observed an increase in ionized calcium-binding adapter molecule 1 (IBA1, 1:200; Wako Pure Chemical Industries, Osaka, Japan) immunoreactivity adjacent to the lesion. These results thus revealed that astrocytic and microglial reactivity in *Microcebus murinus* persist 3 months following SCI. Interestingly we identified a pronounced increase in microglia/macrophage reactivity coinciding with a hyper-intense MRI signal within the ipsilateral dorsal funiculus rostral to the lesion.

Perspective: We have developed and characterized a novel model of SCI in a small non-human primates. We are now deepening our evaluation of the functional recovery of *Microcebus murinus* after SCI. In particular, behavioral assessments will be extended focusing on the grip test that can accurately discriminate fine motor impairments and detailed toe movements. In this regard, we are setting up a video monitoring analysis of *Microcebus murinus* along 3 axes while they are climbing on a scale consisting of bars of different diameters. To reliably quantify the precise force developed by the limbs, a bar with sensors detecting the force exerted by each hind limb may be used. This would allow an objective measurement of the force expressed in Newton as compared to a more subjective scoring based only on visual evaluation. A recent study has used a small iron bar mounted on a piezo-electric force to measure the pull strength of *Microcebus murinus* (Thomas et al., 2016). In this test, lemurs gripped a dowel with their hands and were then pulled away horizontally from the dowel. Peak forces in the horizontal direction were then recorded. An adaptation of this test could permit to also analyze the grip force of the hind limbs following SCI and thus to evaluate outcomes of a given therapeutic strategy. Another line of development is to evaluate sensory functions such as loss of sensation, enhanced abnormal sensation and neuropathic pain that is a common complication of SCI. An approach is to adapt the von-Frey filament test to *Microcebus murinus* following SCI. This test had been successfully used to evaluate responses to peripheral neuropathy following ligation of a spinal nerve in *Macaca fascicularis* (Palecek et al., 1992). Moreover, sensory changes addressed by von Frey test in non-human primate is currently one of the subject of studies developed by the California Spinal Cord Consortium. A simpler method, may be as for human to use different types of stimulation like light touch and pinprick to score responses of the primate (retraction or not of the stimulated hind limb).

In the model that we have recently developed, we used T2-weighted MRI (Multi Echo Multi Slices acquisition protocol) for *in vivo* and *ex vivo* acquisitions. However, to illustrate the mini atlas of *Microcebus murinus* spinal cord, we used *ex vivo* diffusion-weighted-MR axial images (dw-MRI) acquired using two sequential spin-echo multi-slices protocols. *Ex vivo* diffusion MRI allows a better discrimination between the injured and intact tissue due to a higher signal-to-noise-ratio compared to *ex vivo* T2-weighetd images. The use of diffusion-weighted sequences should thus not only permit to better discriminate grey and white matters but also to measure more accurately the evolution of damage tissues over time. A future objective could be to correlate the percentage of lesion with the functional recovery rate.

Finally, from a mechanistic point of view, we will also analyze if a sub-population of astrocytes express markers of neuronal progenitors as we have recently identified in mice (Noristani et al., 2016).

Conclusion: As a conclusion, the new model that we have recently developed in *Microcebus murinus* can be used to promote translational research on SCI and represents an alternative to larger primates. Improvements in the follow-up after injury such as new tests measuring the fine motor movement and pull strength of the hind limbs after SCI, sensory function recovery and *in vivo* diffusion-weighted MRI sequences in this model will contribute and complement our understanding of SCI pathophysiology. Ultimately, it will be used for the development of therapeutic strategies to enhance functional regeneration following SCI.

This work was supported by the patient organizations "Verticale" and "Demain Debout Aquitaine".

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***Accepted:** 2018-02-23*

***doi:** 10.4103/1673-5374.228721.*
***Plagiarism check:** Checked twice by iThenticate.*
***Peer review:** Externally peer reviewed.*

Open peer review reports:
***Reviewer 1**: Tania Cristina Leite de Sampaio e Spohr, Instituto Estadual do Cérebro Paulo Niemeyer, Brazil.*
***Comments to authors:** The manuscript describes the use of the non-human primate model Microcebus murinus as a promising model of spinal cord injury. They also describe some results they have working with this model. The authors also describe shortly why the use of Macaca fascicularis and Callithrix jacchus has some limitations, as non-human primate model for SCI.*
***Reviewer 2:** Carsten Theiss, Ruhr-Universitat Bochum, Germany.*

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