A longitudinal quantitative MRI study of BSCB permeability after Peripheral nerve injury in mice

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Introduction. Peripheral nerve injury (PNI) is debilitating and is estimated to affect 3-8% of people worldwide [1]. Emerging evidence suggests that PNI alters the integrity and increases the permeability of the blood-spinal-cord-barrier (BSCB) [2]. Following the insult, large molecules and immune cells that are normally blocked are able to infiltrate the spinal cord. The pain persists and become chronic even after BSCB returns to a normal level. The dysfunction of the permeability of the barrier may contribute to neuropathic pain hypersensitivity. Dynamic contrast-enhanced MRI has been shown to be a powerful tool to detect leakage of the BSCB [3]. The aim of this study is to evaluate if a non-invasive imaging modality such as MRI could detect small changes in the BSCB permeability longitudinally after PNI in mice.

Methods. Animal model: Spared nerve injury (SNI) [4], a model of PNI, was performed in adults CD-1 male mice (n=8 SNI and n= 6 naïve). SNI consists of a partial denervation of the sciatic nerve from the tibeal peroneal area. Data acquisition: Mice were imaged 6hr, 24hr, 3 days and 7 days post-injury with a multi-channel 7.0T MRI scanner (Varian Inc., Palo Alto, CA). Customized phantoms, two calibrated glass micro capillaries containing 1% Gd-DTPA in agar, were placed with each mouse. Animals received an injection of Gd-DTPA (1.5 mmol/kg) intraperitoneally for each imaging session. Mice were injected with Evans Blue and sacrificed at the end of the study. Imaging Parameters: 2D T1-weighted coronal images were acquired using a spin echo sequence with 100 um in plane-resolution, TR = 500 ms, TE = 9 ms, 3 mm slice thickness and nt = 8. Data processing: ROIs were placed outlining the entire spinal cord. Quantitative measurements of signal intensity were determined by normalizing the spinal cord intensity to the average intensity of the two phantoms included in each coil.

Result. 2D images were obtained of SNI and naïve mouse spinal cords at both thoracic and lumbar regions (Figure 1). The graph (Figure 2) represents the normalized spine intensity over the time course. We observed greater normalized intensity within the spine from injured (SNI) mice in comparison to naïve mice after 24 hours. The increase is transient and appears to return to normal level by 7 days. This is consistent with a cross-sectional study using Evans Blue in rats with PNI [2]. A direct correlation was found between the normalized spine intensity and the levels of Evans Blue in the spinal cord. *In vivo* MR Imaging may provide an excellent alternative to a tracer such as Evans Blue, which requires sacrificing the animal at each time point in order to study barrier damage.

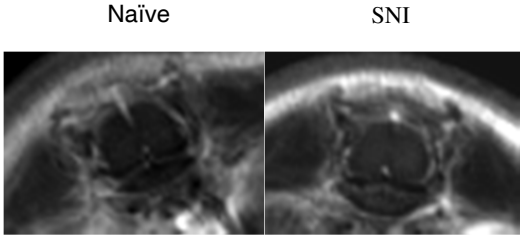

Figure 1: Representative coronal MRI scans through lumbar spinal cord

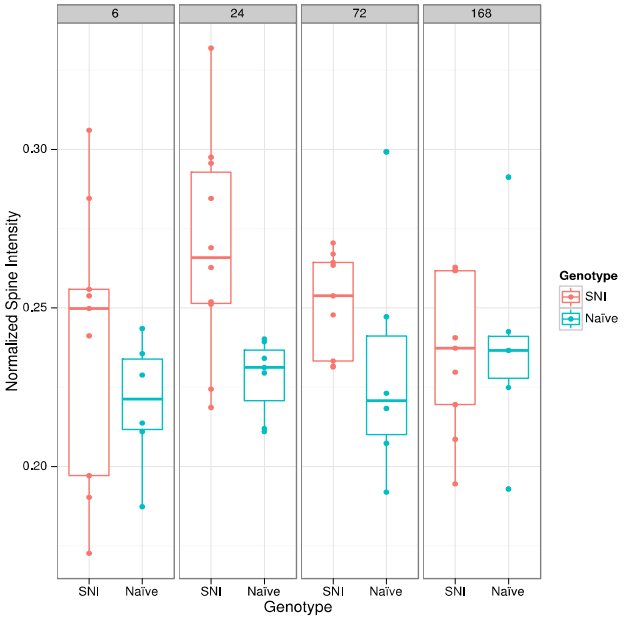

Figure 2. Box and whisker plots showing normalized spine intensity over time course (in hours) in mice with SNI (red) and Naïve (blue)

Discussion. The longitudinal study and the use of Gd contrast agent is a promising approach to assess the BSCB permeability and detect subtle changes with PNI. We are interested to use a non-invasive tool for monitoring the BSCB breaking in several mouse models for pain. Our future work may provide a better understanding on the mechanism responsible for the production of neuropathic pain. Furthermore, our study will contribute to the progress on the genetic studies in pain.

References. [1] Hughes et al., British Medical Journal (2002), 324:466-469., [2] Beggs, Molecular Pain (2010), 6:74., [3] Bilgen, Magnetic Res. in Medicine (2001) 46: 1099-1106., [4] Decosterd et al., Pain (2000) Aug:87 (2)149-58.