# MRI of Focal EAE Progression in a Rat Model following VEGF-induced Opening of the Blood Brain Barrier

Jasper Chen[1,2], Melina Jones[3], Miroslaw Janowski[2,4], Jiadi Xu[5], Michael Levy[3], Jeff WM Bulte[1,2], and Piotr Walczak[1,2]

[1]Institute for Cell Engineering, Johns Hopkins University, Baltimore, MD, United States, [2]Division of MR Research, Russell H. Morgan Department of Radiology and Cell Imaging Section, Johns Hopkins University, Baltimore, MD, United States, [3]Department of Neurology, Johns Hopkins University, Baltimore, MD, United States, [4]NeuroRepair Department, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland, [5]F. M. Kirby Research Center, Kennedy Krieger Institute, Baltimore, MD, United States

**Target Audience:** This work will be of interest to researchers and clinicians working in the field of neurology and neuroscience, in particular those interested in myelin diseases such as multiple sclerosis (MS).

**Purpose:** Experimental autoimmune encephalomyelitis (EAE) is a widely used animal model of MS with disseminated, demyelinating lesions localizing to the white matter of the brain stem and spinal cord. In contrast to cerebral lesions in patients, highly variable distribution of lesions, which primarily localize within the brain stem and spinal cord, is one of the main disadvantages of current EAE models[1]. Our goal was to develop a clinically relevant, highly reproducible EAE model with robust MRI readouts for the evaluation of disease course and assessment of novel therapeutic strategies.

**Methods:** Seven week-old female DA rats were injected with a 100 μl emulsion of 6.25 μg myelin oligodendrocyte glycoprotein (MOG 1-125) and 50 μg Complete Freund's Adjuvant into the tail-base to immunize the animals against myelin. Three weeks after immunization, 0.75 μg vascular endothelial growth factor (VEGF) in 1.5 μl phosphate buffered saline was stereotaxically injected into the internal capsule (i.c.) of the rat brain to open the blood brain barrier (BBB). All imaging was performed using a high field Bruker 11.7T MRI animal scanner. BBB opening was detected *in vivo* using a T1-weighted, MP-RAGE sequence with 0.5 mmol/kg gadolinium-DTPA contrast enhancement, injected intraperitoneally. For Evans blue staining, 0.8 ml, 4% Evans blue was injected into the femoral vein and the rats was sacrificed 4 hours later. Goat anti-rat IgG was used for immunofluorescence (IF) detection of intraparenchymal immunoglobulins. Inflammation was monitored by T2 decay calculated from a multi-slice, multi-echo (MSME) T2-weighted MRI.[2] H&E staining was performed using standard protocols. Staining for ED1 was used for IF detection of activated microglia and macrophages. Magnetization transfer ratio (MTR) was used as a MRI marker for myelination status.[3] Eriochrome staining was used for detection of myelin. Staining for neurofilament (NF) and myelin basic protein (MBP) was used for IF detection of demyelinated axons.

**Results:** Injection of VEGF into the brain parenchyma resulted in disruption of BBB as shown by gadolinium enhancement T1-weighted MRI (**a**). Longitudinal imaging demonstrated that BBB is disrupted one day post-VEGF, with a gradual return to baseline over a period of two weeks (**c**). Evans Blue, a histological marker for BBB disruption accumulated at the site of injection; however the region was much smaller compared to that detected on MRI. IF for rat IgG showed leakage of blood immunoglobulins into the brain with the lesion size corresponding to Evans Blue (**b**). An inflammatory response characteristic for demyelination was detected on T2-w MRI as a hyperintense region at the VEGF-injected hemisphere (**e**). Longitudinal MRI demonstrated brain inflammation following BBB opening, with a peak around 5 days post-VEGF (**d**). Hyperintensity was no longer detected on day 14, consistent with resealing of the BBB. H&E staining showed a significant infiltration of immune cells, particularly concentrated around blood vessels (**f**). IF for ED1 showed substantial activation of microglia and infiltration by macrophages, which is associated with MS pathology. MTR imaging detected reduction of hyperintensity within the VEGF-injected i.c. indicative of myelin loss (**i**). Longitudinal MTR imaging (**g**) shows greatest reduction on day 4, similar course to T2-w inflammation imaging (**d**). Histological stain for myelin (eriochrome) showed dramatic myelin loss in i.c seven days post-VEGF. IF for axonal NF (green) and myelin MBP (red) showed many demyelinated axons (**h**). The demyelination was primarily localized around blood vessels. MTR hyperintensity levels returned to normal at day 14, suggestive of remyelination (**j**). Eriochrome showed restoration of myelin. The NF and MBP staining coincided, showing no detectable demyelinated axons. A strong GFAP staining was seen around the repaired lesion, indicative of astrocyte activation and MS pathology.
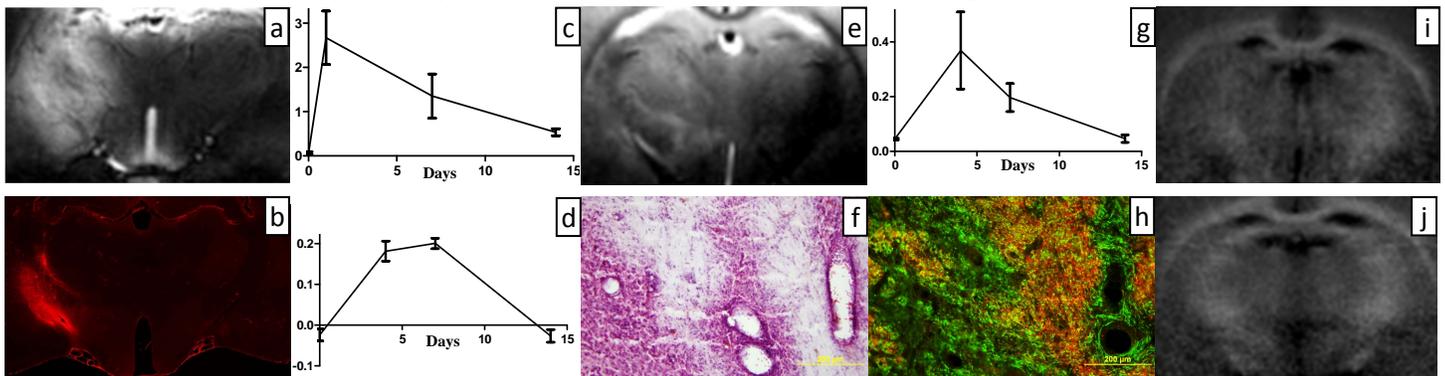


**Discussion:** We used stereotaxic targeting to produce focal lesions, giving control over lesion size and location, reducing variability. We demonstrated that each event in the pattern of lesion formation (BBB permeabilization, inflammation, and demyelination) can be monitored by specific MRI sequences. Precise control over time and location of lesion formation makes our model ideal for MRI evaluation of therapies for not only the final disease state but also the entire lesion-forming process.

**Conclusion:** Our EAE model is more clinically relevant than currently available models. Inflammatory/demyelinating lesions as monitored by *in vivo* MRI and histology were robust but rapidly repaired within two weeks. Future work will include adjusting the model parameters to achieve a longer disease duration for evaluating therapies aimed at chronic MS.

**References:** [1] M Sasaki, et al. Glia. 2010, 58. [2] DH Miller, et al. Brain. 1998, 121(1). [3] V Dousset, et al. Radiology. 1992, 182(2).