MRI-guided fluorescence imaging of glial reactivity in chronic neuropathic pain

S. C. Davis[1], L. H. Treat[1,2], E. A. Romero-Sandoval[2,3], K. S. Samkoe[1], B. W. Pogue[1], and J. A. DeLeo[2,3]
[1]Thayer School of Engineering, Dartmouth College, Hanover, NH, United States, [2]Department of Anesthesiology, Dartmouth College, Hanover, NH, United States, [3]Department of Pharmacology and Toxicology, Dartmouth College, Hanover, NH, United States

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Background

Glial reactivity plays an important role in the biochemical processes associated with acute and chronic pain as well as neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and multiple sclerosis. Quantitative imaging of glial reactivity *in vivo* would be an important innovation for investigating and deploying new treatment strategies that target glial mechanisms. This study examines the potential of MR-guided fluorescence molecular tomography (MRg-FMT) to image astrocytic changes in the brain and spinal cord of rats after peripheral nerve injury. Fluorescence measurements acquired from within the magnet bore were combined with high resolution MRI data to recover three-dimensional images of fluorescence activity in the tissue. In this hybrid imaging paradigm, segmented MR images provide a three-dimensional structural template upon which fluorescence activity is quantified. This synergistic approach has been shown to improve imaging performance in a variety of optical imaging applications, but has not previously been applied to glial reactivity imaging *in vivo*.

Methods

Chronic pain model and imaging agent

Seven days prior to imaging, male Sprague-Dawley rats underwent surgical transection of the L5 peripheral spinal nerve while anesthetized. Control animals underwent sham surgeries without L5 transection or no surgery. The optical molecular probe consisted of a near-infrared imaging dye (IRDye 800CW, LI-COR) conjugated to anti-Glial Fibrillary Acidic Protein (GFAP), a marker for astrocytes.

Imaging system

A unique multi-spectral fluorescence molecular tomography scanner(1) coupled to a Philips Achieva 3T whole body scanner (Figure 1[a]) was used to quantify GFAP status *in vivo*. The MR-optical animal interface, shown in Figure 1(b), was designed to position optical fibers around the rat's head and in two rows along the spine. This system was fixed inside a standard MRI head coil for simultaneous optical-MRI acquisition, facilitating perfect co-registration of MR and optical data.

Scanning and image recovery

Animals were administered the GFAP-targeted NIR fluorophore via lumbar puncture 48 hours prior to imaging. MRg-FMT scans of anesthetized animals could be completed within 30 minutes. After scanning, brain tissue, cerebral spinal fluid, and spinal cord tissue were surgically harvested and imaged on a Licor Odyssey tissue specimen scanner. Recovery of fluorescence activity began by segmenting T2-weighted image stacks of the rat head into brain and non-brain regions. These segmented tissue regions formed three-dimensional spatial *a priori* templates used to guide the optical image recovery algorithms, a process illustrated in Figure 1 (c) – (e). The optical algorithms are derived from the diffusion approximation of photon transport through tissue and are used to recover values of fluorescence activity in the brain tissue, a quantity associated with GFAP expression.

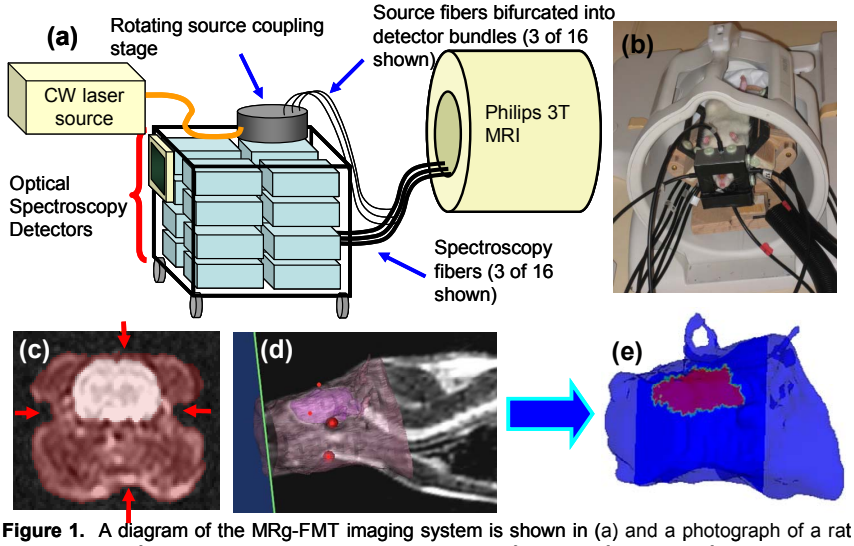

Figure 1. A diagram of the MRg-FMT imaging system is shown in (a) and a photograph of a rat in the optical interface in an MR head coil in (b). In this configuration, four optical fibers surround the head, as illustrated in a coronal slice of the head in (c), and ten fibers run along the spine in two rows of five. T2-weight MR images of the rat were segmented in brain and head and head regions (d). This three dimensional spatial template was combined with the optical data to recover fluorescence activity in the head (e).

Results and Discussion

Examples of MRg-FMT images of GFAP expression in the rat head are shown in Figure 2 for animals that underwent sham surgery (a), no surgery (b), and L5 nerve transection (c). These images show a dramatic increase in fluorescence activity in the brain of the injured animal while animals that underwent sham surgery or no surgery produced low levels of fluorescence activity. Ex-vivo analysis of harvested tissue confirmed elevated levels of fluorescence activity in injured animals and lower fluorescence in the brains of control and sham surgery animals.

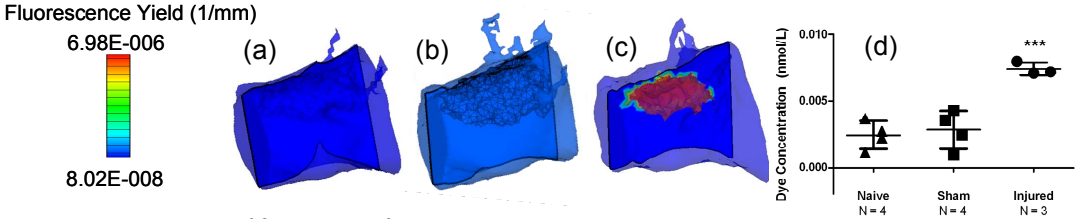

Figure 2. Examples images of GFAP-targeted fluorescence activity in animals with sham surgery (a), no surgery (b), and L5 nerve transection (c). The fluorescence intensity of harvested brain tissue *ex-vivo* scans is quantified in (d).

Conclusion

Ex-vivo analysis of brain and spinal cord slices indicate excellent specificity of the GFAP-targeted fluorophore to injured animals. Preliminary MRg-FMT images of rat brains reflect the *ex-vivo* results, demonstrating strong potential for discriminating GFAP status *in vivo*. Reliable quantification of GFAP activity in the brain would be a useful tool for developing treatment strategies based on modulating glial responses to neuropathic pain.

References1

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