

## VISUALIZATION OF IMMUNE CELL DYNAMICS IN MOUSE BRAIN WITH 11.7 T MRI

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### **Introduction:**

Macrophage and microglia in the central nervous system (CNS) play an important role in the neuroinflammatory disease, such as multiple sclerosis and ischemic injury. Even in the normal (intact brain), those immune cells actively reached out the immune response for plasticity of neuronal environment [1]. We can say with fair certainty that immune cells in the CNS may contribute the surveillance of the brain environment [1,2]. The dynamical behavior of immune cells in intact/injured CNS has not been well characterized with imaging technique. Non-invasive monitoring of immune cells before/after neuroinflammatory conditions may lead to a greater understanding of the mechanistic underpinning of both CNS injury and repair. We perform 11.7 T MRI to evaluate the potential for non-invasive monitoring of immune cells in the CNS and try to delineate the recruitment and migration of immune cells with or without inflammation in mouse brain using intravenous superparamagnetic iron oxide particles as a contrast medium.

### **Methods:**

Male C57BL6J mice (8-12 week-old) were split into two experimental groups; LPS (inflammation) and saline (normal control) groups. Mice received intraperitoneal administration of 100  $\mu$ L LPS (100  $\mu$ g/ml) or saline, respectively. All mice were anesthetized with isoflurane in room air via a nose cone and maintained a constant respiration rate of  $60 \pm 10$  breathes/min during each MRI session. The mouse head was placed in a 15-mm inner diameter transmit/receive volume RF coil (m2m imaging). MRI was conducted on an 11.7 T vertical bore imaging system (AVANCE II, Bruker). Following baseline scans at 3 days after intraperitoneal administrations, 100  $\mu$ L of super paramagnetic iron oxide nanoparticles (SPIOs; Resovist, Schering) were injected into the tail veins of anesthetized mice. After that, mice were allowed to wake up and were placed back in their cage. At 24-, 48- and 168-hr post SPIOs injection, the mice were anesthetized again, and  $T_2^*$ -weighted MRI was acquired with 2D-FLASH sequence (TR/TE = 100/4 ms).

### **Results and Discussion:**

Surprisingly, several tiny non-specific black spots induced by SPIOs were found even in the normal control brain at 24- and 48-hour post SPIO injection (see Figure 1, top row). These spots were reduced at 1 week. In the LPS-induced inflammation group, a large amount of black spots was found in the brain at 24-hour post SPIO injection, and the quantity of spots increased up to 48-hour post injection (see Figure 1, bottom row).

It is considered that the blood-brain barrier (BBB) prevents the passage of many substances into the brain or spinal cord, including ionized water-soluble molecules with molecular weights greater than 500 Da [3]. SPIOs themselves may be blocked by BBB. However, the SPIOs that were incorporated in the phagocytic cells can pass through the BBB, because macrophages and microglia have a natural ability to traverse the intact and compromised BBB [4]. Therefore, the hypointense on SPIO-enhanced  $T_2^*$ -weighted images represent the existence of phagocytic cells in the brain.

### **Conclusion:**

We report that intravenous SPIO-enhanced MRI has a great possibility for visualizing the recruitments and migrations of immune cells in mouse brain *in vivo*, even in the normal control. Immune cells in the CNS markedly increased in the inflammatory conditions with 11.7 T MRI. Our technique could contribute to establish the novel estimation method for treating brain injury and also maintenance in normal condition that target immune cells.

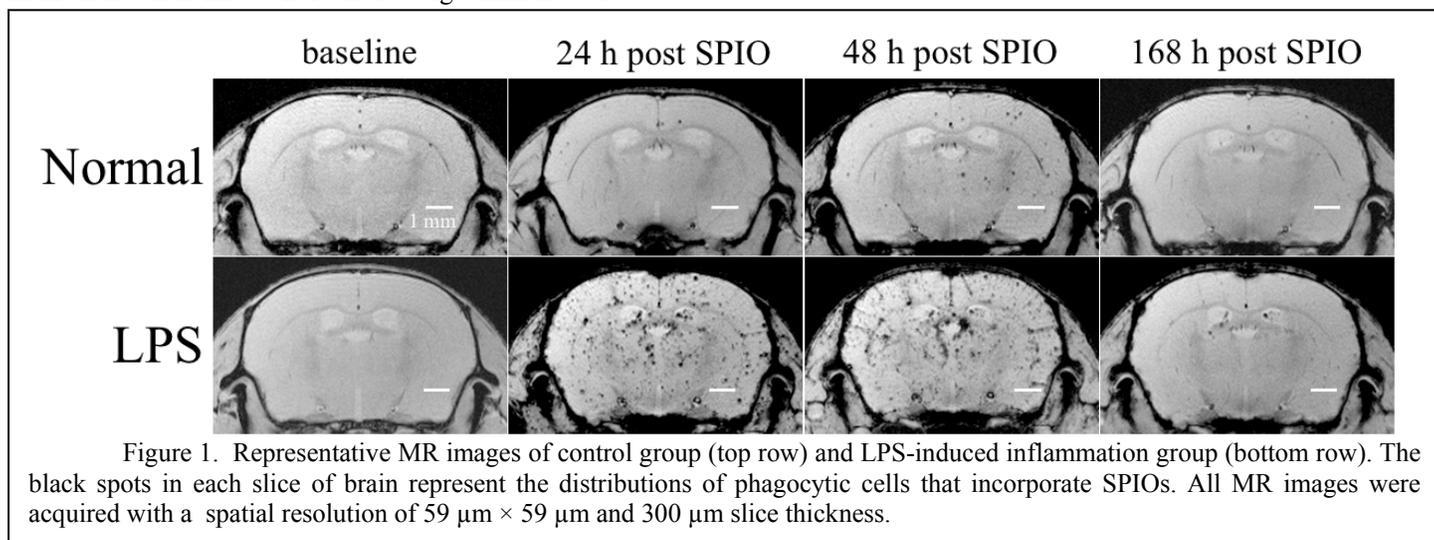


Figure 1. Representative MR images of control group (top row) and LPS-induced inflammation group (bottom row). The black spots in each slice of brain represent the distributions of phagocytic cells that incorporate SPIOs. All MR images were acquired with a spatial resolution of  $59 \mu\text{m} \times 59 \mu\text{m}$  and  $300 \mu\text{m}$  slice thickness.

**References:** [1] Wake *et al.*, J Neurosci, 29: 3974-80 (2009), [2] Wilson *et al.*, J Clin Invest, 120: 1368-79 (2010), [3] Pardridge. Neuron, 36: 555-8 (2002), [4] Hickey. Semin Immunol, 11: 125-37 (1999)