

## **In Vivo MRI**

### **Animal:**

The *in vivo* MR imaging was conducted while maintaining the animal in a supine position on an imaging stretcher under anesthesia with 2.0% isoflurane (Abbott Laboratories, Abbott Park, IL, USA) in an oxygen and air mixture. During the imaging, the heart rate, SpO<sub>2</sub>, and rectal temperature were regularly monitored to manage the animal's physical condition.

### **Scanner and coil system:**

MRI was performed using a 9.4 T BioSpec 94/30 (Bruker Optik GmbH, Ettlingen, Germany) unit and a transmitting and receiving coil with an 86-mm inner diameter.

### **Imaging:**

#### T1 weighted imaging

Magnetization Prepared - RApid Gradient Echo (MP-RAGE) was used with TR = 6000 ms, TE = 2 ms, flip angle (FA) = 12 degrees, number of average (NA) = 1, inversion time = 1600 ms, voxel size = 270 × 270 × 540 μm, and scan time = 20 min.

#### T2 weighted imaging

Rapid Acquisition with Relaxation Enhancement (RARE) was used with TR = 4000 ms, TE = 22 ms, RARE factor = 4, FA = 90 degrees, NA = 1, voxel size = 270 × 270 × 540 μm, and scan time = 7 min 24 sec.

#### Diffusion weighted imaging

Spin-echo echo planar imaging was used with TR = 3000 ms, TE = 25.6 ms,  $\delta$  = 6 ms,  $\Delta$  = 12 ms, b-value = 1000 and 3000 s/mm<sup>2</sup> in 30 and 60 diffusion directions, respectively (plus 4 b0 images), number of segments = 6, FA = 90, NA = 3, voxel size = 350 × 350 × 700 μm, and scan time = 90 min. Diffusion metrics were created by DTI model, and diffusion fiber connectome was created by the constrained spherical deconvolution (Tournier et al., 2004).

#### Resting state functional imaging

Gradient recalled echo echo planar imaging was used with TR = 1500 ms, TE = 18 ms, number of shots = 1, FA = 40, NA = 1, number of repetition = 400, voxel size =  $500 \times 500 \times 1000 \mu\text{m}$ , and scan time = 10 min.

## **Ex Vivo MRI**

### **Animal:**

The animal was perfusion-fixed with 4% paraformaldehyde (PFA), the brain was dissected out of the cranium, and soaked in PFA for *ex vivo* imaging. During *ex vivo* imaging, the brain was wrapped in a sponge and soaked in fluorine solution, which exhibits no signal on MR images, in a plastic container. Vacuum degassing was performed for artifact reduction. PFA for the fixation was replaced with fresh solution weekly to maintain the effect of fixation.

### **Scanner and coil system:**

MRI was performed using a 9.4 T BioSpec 94/30 (Bruker Optik GmbH, Ettlingen, Germany) unit and a transmitting and receiving solenoid type coil with an 28-mm inner diameter.

### **Imaging:**

T2 weighted imaging

Rapid Acquisition with Relaxation Enhancement (RARE) was used with TR = 10000 ms, TE = 29.3 ms, RARE factor = 4, FA = 90 degrees, NA = 16, voxel size =  $100 \times 100 \times 200 \mu\text{m}$ , and scan time = 3 hour 20 min.

Diffusion weighted imaging

Spin-echo echo planar imaging was used with TR = 4000 ms, TE = 28.4 ms,  $\delta = 7 \text{ ms}$ ,  $\Delta = 14 \text{ ms}$ , b-value = 1000, 3000 and 5000  $\text{s/mm}^2$  in 128 diffusion directions each (plus 6 b0 images), number of segments = 10, FA = 90, NA = 2, voxel size =  $200 \times 200 \times 200 \mu\text{m}$ , and scan time = 6 hour 39 min. Diffusion metrics were created by DTI model, and diffusion fiber connectome was created by the high angular resolution diffusion-weighted MRI (Frank LR, et al., 2002).