Corticospinal Tract Tracing In Mouse Using Manganese-Enhanced Contrast

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Abstract

Application of manganese-enhanced magnetic resonance imaging was expanded to trace the corticospinal tract (CST) in live mouse. Motor cortex of mouse was injected with manganese bilaterally and stimulated electrically to facilitate uptake of manganese into the cortical neurons and to increase its axonal transport distance. Next day, brain and spinal cord of mouse was imaged using spin echo and inversion recovery spin echo sequences. Optimal strategies and experimental procedures enable to achieve a clear visualization of the anatomical and structural organization of the CST within the brain and spinal cord. Considering that mouse is increasingly used as an animal model of spinal cord injury, the availability of this new neuroimaging tool is anticipated to open up new opportunities in this line of research, especially in terms of evaluating axonal damage, CST reorganization and intervention strategies.

Keywords: Manganese, manganese-enhanced magnetic resonance imaging, corticospinal tract, spinal cord, spinal cord injury, mouse

INTRODUCTION

In the central nervous system (CNS), the corticospinal tract (CST) consists exclusively of motor axons that originate from cell bodies in the motor areas of the cerebral cortex and project anterogradely through the spinal cord (SC). These axons transmit signals from motor neurons that control a variety of voluntary movements. The function and organization of CST are known to exhibit differences between species (20). Nevertheless, in all species, spinal cord injury (SCI) damages CST and causes paralysis below the site of the injury. Thus, the CST remains the main focus in neuronal regeneration and reorganization studies in experimental SCI research (2,15,18). Typically, the CST and its sprouting axonal fibers are visualized using neuronal tracers evaluated with histological analysis (3,16,24,25). These traditional tract tracing methods are
invasive and require end-point tissue collection and processing for histology. Alternatively, a number of in vivo neuroimaging tools have been developed to probe the CNS under normal and pathological conditions. With these tools, one can monitor the progression of the underlying tissue pathology longitudinally without disturbing its natural course and use each subject as its own control. Among these approaches, magnetic resonance imaging (MRI) modalities have emerged to provide non-invasive visualization in anatomical, structural and functional studies of SC in humans and animals. Until recently, distinguishing CST alone was not possible on MR images because of the lack of endogenous MR contrast between this tract and its surrounding tissue. To facilitate the recognition of the CST on MR images, paramagnetic manganese (Mn) was introduced as signal enhancing contrast agent. A single-focal, intracortical injection of Mn into the rat’s motor cortex was shown to label the descending cerebral pathways in the rat brain on Mn-enhanced MRI (ME-MRI). More recently, we have shown that electrically stimulating the cortex increases the transport, uptake and accumulation of Mn in CST, and allows better delineation of this tract in rat SC. More importantly, this approach offers new possibilities for detecting the continuity and connectivity of the CST fibers in injured SC.

The utilities of ME-MRI-based CST tracing have so far been demonstrated only in experiments with normal rat or rat model of SCI. However, mouse is increasingly employed in SCI research. The availability of transgenic and knockout mouse strains offer new opportunities in terms of understanding the pathophysiological roles of specific genes and defining signaling pathways in SCI. Molecular and cellular studies of SCI are conducted to comprehend neuronal plasticity in injured SC and formulate effective therapies for preserving neuronal tissue or promoting regeneration to improve the neurofunctional outcome from SCI. Therefore, it is quite appealing to test the possibility of visualizing CST in mouse using ME-MRI and investigate the potential merits of this approach in detecting viable CST fibers in injured mouse SC. Because mouse is smaller in size and its central nervous system is shaped slightly different than rat, Mn-based tracing strategies developed for rat can not be applied directly to image the mouse CST. In this paper, we give experimental procedures and modifications required for achieving optimal performance in tracing mouse CST using ME-MRI. We give information on the location, dose and volume of the Mn injection, describe MRI protocols for sensitively visualizing the Mn-labeled corticospinal pathway, and provide examples to demonstrate the feasibility of imaging CST in live mouse.

**METHODS**

C57BL/6 mice weighing between 25 and 32 g were used for the experiments under the protocols approved by the University of Kansas Medical Center Institutional Animal Care and Use Committee and executed in accordance with the Guide for the Care and Use of Laboratory Animals.

**Surgeries**

Mouse was anesthetized with ketamine hydrochloride injected intraperitoneally at an initial dose of 150 mg ketamine/kg. Additional doses of 5-20 mg/kg were delivered intramuscularly as needed (1 – 2 times) during the entire surgery. After fixing the head in a stereotaxic frame (Kopf Instruments, Tujunga, CA), a midline incision was made on the scalp from approximately 2.5 mm rostral to the bregma to 2.0 mm caudal to the lambda, and the skin was retracted with hemostats. Coordinates of the motor cortex were identified from a published mouse brain atlas. To access the motor cortex, two 1.0 mm burr holes were drilled into the skull bilaterally at 1.5 mm rostral to the
bregma and 1.0 mm lateral to the midline using a 1 mm diameter trephine bit attached to a dental drill (Model SH28, OSADA Inc., Los Angeles, CA). An additional third craniotomy of 1.0 mm in size was performed on one side of the skull at location 0.5 mm rostral to the lambdoid suture and 1.5 mm lateral to the midline.

**Mn injection into motor cortex**

A 1-µL Hamilton syringe was inserted into a glass micropipette prepared from 1.0 mm outer diameter capillary tubing by a vertical pipette puller (Model 700D, Kopf Instruments, Tujunga, CA). The syringe was filled with a solution of 1 M MnCl₂ and mounted on a micropositioner attached to the stereotaxic frame. As the syringe was lowered, the tip of the micropipette was inserted at a 35º angle to the pial surface and 0.8 mm in length (0.3 mm in depth) into the cortex in the first burr hole. After being propelled to deliver 0.2 mL of the solution over a period of 5 min, the syringe was left in place for another 5 min prior to withdrawal to prevent backflow of solution. These steps were repeated to deliver the contrast solution through the second burr hole.

**Electrical stimulation of motor cortex**

After draining any fluid accumulated in the burr holes by a cotton swab, electrical stimulation was applied by placing a 0.2 mm diameter stainless steel electrode onto the dura through the first burr hole, and inserting and gluing a reference electrode to touch the dura through the third craniotomy. The electrodes were connected to a constant current stimulator (Model DS7, Digitimer Ltd., Heartfordshire, England). Trains of biphasic stimuli were generated by a Grass S48 stimulator (Grass Medical Instruments, Quincy, MA) at 100 Hz and a 5 sec on-off cycle. Visible motor responses by the forelimb, hindlimb or tail were evoked at 0.8-1.1 mA and maintained for 90 min. Next, the stainless steel electrode was removed from the first burr hole and placed into the second burr hole to stimulate the cortex contra laterally using the same stimulation paradigm. After finishing this process, the burr holes were glued, skin was sutured and the mouse was left to recover in its cage. The surgeries, injection and stimulation were completed in 4 - 5 hr.

**Magnetic resonance imaging**

The next day, approximately 15 h after the operation, the mouse was scanned using a 9.4 T horizontal Varian scanner (Varian Inc., Palo Alto, CA) under the inhalation of isoflurane anesthesia (7-10). Spontaneous anesthesia induced by 4% isoflurane was followed by delivery of an air mixture with 1.0% isoflurane and 30% oxygen through a nose mask. The mouse was placed in a Plexiglas holder and positioned into a 38 mm inner diameter volume coil for imaging. The respiration was monitored and the acquisition was gated by an MRI compatible monitoring and gating system (Model 1025, SA Instruments, Inc., Stony Brook, NY). Body temperature of the animal was kept at 37 ºC by circulating warm air with 40% humidity using a 5 cm diameter plastic tube fitted at the back door of the magnet bore.

Anatomical images were acquired from brain and SC using spin-echo (SE) sequence with the parameters values T/E = 4000/10 ms, number of excitations (NEX) = 2 and slice thickness = 1 mm for the axial (image matrix = 128 X 128, field of view = 11 X 14 mm²) and 0.5 mm for the sagittal (image matrix =256 X 128, field of view = 32 X 12 mm²) visualization. For ME-MRI, T1-weighted imaging images were acquired using an inversion recovery spin echo (IR-SE) sequence with the parameters T/E/T = 2000/14/500 ms. Sagittal slice thickness was 2 mm, but the thicknesses of the axial slices varied from 1 mm to 2 mm and NEX varied from 4 to 10, depending on whether the brain or SC was imaged. After these scans, the mouse was removed from the magnet bore, euthanized by 4% chloral hydrate at 400 mg/kg and its vertebral body was dissected. The excised sample with intact spine was imaged ex vivo at
room temperature using an inductively coupled surface coil centered at the injury epicenter (10-12). Anatomical data were obtained from the spine and SC in axial views using $T_R/T_E = 4000/10$ ms, image matrix = 128 X 128, field of view = 5 X 5 mm$^2$, slice thickness = 1 mm and NEX = 2. The acquisition was repeated with the same slice parameters using the IR-SE sequence with $T_R/T_E/T_I = 2000/17/500$ ms and NEX = 8.

RESULTS AND DISCUSSION

The experimental procedures described above for the surgeries, Mn-injection and electrical stimulation were in general feasible as applied to the mouse. Because the brain and SC in mouse are smaller than those in rat, the experiments on mouse required better skills and more practices to position the micropipette in the motor cortex and to deliver an adequate volume of Mn, as compared to our previous studies performed on rats (4-6). In some situations, we experienced difficulties which led to repeating the experiment multiple times on different animals. The difficulties were largely related to injecting Mn into the motor areas of the brain which were only about 1 mm in depth and especially challenging in positioning the micropipette tip. Even exercising careful manipulations of the micropipette, we still experienced that in some cases the tip easily over-passed the cortex and Mn was delivered into the subcortical areas. In other cases, the tip was inserted properly into the cortex, but the contrast solution was delivered in volumes lesser or larger than the intended amount of 0.2 mL because of the backflow of the solution. We also experienced inconsistency associated with volume and tip positioning during bilateral injection. Sometimes, one site of the brain received well localized Mn in a right amount, but the contra lateral site either had the injection missing the cortex or received an inadequate volume of solution. These problems were difficult to notice at the time of the injection, but clearly recognized when the animal was imaged.

The use of Ketamine anesthesia allowed the electrical stimulation produce observable contra lateral motor movements in the hindlimbs and changes in the breathing pattern of the animal. These responses ensured that the calcium channels opened in an activity-dependent fashion and hence the uptake of Mn was facilitated into the cell bodies of the motor neurons.

The practical issues described above were solved by improved skills obtained through repeated experiments. Subsequently, the scans with the SE and IR-SE sequences and the given imaging parameters produced high quality data. Representative SE and IR-SE images in Fig. 1 show the brain and SC of a mouse in sagittal view.

**Figure 1:** Sagittal images of brain and spinal cord of a mouse scanned using a spin echo (SE) and an inversion recovery-spin echo (IR-SE) imaging sequences. (a) SE and (b) IR-SE images were acquired 15 h after the electrical stimulation of the cortex. The slice thickness is 0.5 mm for the SE image and 2 mm for the IR-SE acquisition. Colored lines identified with numerals 1, 2, 3 and 4 denote the slice locations for the images shown in Fig. 2. Arrow head point to the site of the Mn injection and arrows point to the corticospinal tract labeled with Mn.
Given that the bilateral injections were made 1 mm laterally with respect to the midline and the slices centered at the midline were 0.5 and 2 mm thick in the anatomical and ME-MRI images, respectively, the data do not show the exact injection sites in relation to the rostral-caudal direction, but rather the presence of Mn detected within the midline slice after 15 hr of injection. The heterogeneous signal variations within the images (Figs. 1-a and b) represent the distribution of Mn in brain tissue. The hypointense region on the SE image denotes the site of Mn injection in the motor cortex towards the frontal section of the brain (Fig. 1-a). In contrast, the ME-MRI in Fig. 1-b depicts the regions occupied by Mn by hyperintensity over a larger spatial domain including the thalamus. Examining the brainstem and SC on this image closely reveal a thin line of signal enhancement present in the pyramidal tract, which project through the pyramidal decussation into the CST in SC. The CST pathway in mouse was described previously using traditional tracers and histological analysis (22). The contrast features seen in Fig. 1-b were in agreement with this pathway, and ascertained that the labeling of CST with Mn was successful.

The brain and SC of the same mouse were further imaged in axially oriented planes at multiple locations (Fig. 2). The orientation and location of the axial slices were marked by the oblique lines drawn on the reference image in Fig. 1-a. In Fig. 2, the SE images show the details of the brain and SC anatomy in axial views, but can be seen as insensitive in delineating the CST. On the other hand, the IR_SE images (Fig. 2-b) offer contrast enhancement features that could be identified unequivocally in each slice. These enhancements were confined to specific places in the brain along the CST pathway as reported by Paxinos and Franklin (22). Explicitly, the CST initially run down bilateral in thalamus and internal capsule and merged toward the midline in medulla before reached the pyramidal decussation in the brainstem. Then, it changed its course at this junction by crossing to the opposite side and descended in the SC. The distinct region of hypointensity confined to the ventral-most part of the dorsal funiculus of the SC is the place where the CST is known to lie anatomically (16). Finally, the SC was imaged ex vivo at higher resolution after the excision of the spine (Fig. 3). The Mn-labeling of the CST in SC is better appreciated in the IR-SE images in this figure.

Figure 2: Axial SE images (a-d) and the corresponding IR-SE images (e-h). The slice locations for the acquisitions are depicted in Fig. 1-a. Arrows point to the Mn enhancements along the corticospinal tract at the level of thalamus, pyramidal tract, cervical spinal cord and thoracic spinal cord, respectively.
In vivo and ex vivo data presented above collectively demonstrate the feasibility of reliably labeling the CST with Mn and tracing its spatial course within the brain and SC of mouse using ME-MRI. This ability broadens the perspective in neurological research using a variety of transgenic mouse models of SCI and innovative pharmacological interventions. In these experiments, ME-MRI may help us to understand how an injured CST reorganizes and how an applied therapy influences this reorganization and hence improves the recovery from SCI.

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REFERENCES