Timelapse Mn++ Enhanced MRI in the living brain.

E. L. Bearer[1], X. Zhang[2], T. Hiltner[2], J. M. Tyszka[2], R. E. Jacobs[2]
[1]Brown Univ, Providence, RI, United States, [2]California Institute of Technology, Pasadena, CA, United States

Introduction

Manganese-enhanced MRI (MEMRI) allows tracing of neuronal tracts in living animals, which thus offers the potential to observe active transport over time (1, 2). To investigate this potential we used the optic system because its anatomy and transport properties have been well defined. T1-weighted slab images were acquired at 6 min intervals over the course of 2 hr after injection of Mn^{++} into the eye. The rate of Mn^{++} transport was measured by comparing intensity changes along the optic tract. We monitored the toxicity of Mn^{2+} injection on the visual system's response to light by measuring visual evoked potentials (VEP). To assess whether neuronal activity affects Mn^{2+} transport, we compared sighted versus mice with retinal blindness. Time lapse imaging of Mn^{++} transport also reveals details about the mechanism useful for the interpretation of MEMRI applied to tract tracing.

Materials and Methods

Visual evoked potentials were measured before, 3 hours and 24 hours after injection of Mn^{++} into the eye. Sighted C57LB/6 and blind CBA (rd1-/- with retinal blindness) were tested and also analyzed for the rd1 mutation, which causes retinal blindness due to loss of photodetector cells, by PCR. Labview software was used to collect 250 data points at a sample rate of 1kHz recording electrical activity over the visual cortex in response to light stimulation with a flash frequency of 2Hz. Data from 5 repeats were averaged. Retina and optic tracks were examined histologically for numbers and size of axons. For MR, $MnCl_2$ (200 mM) was injected into the vitreous of one eye (0.25 µl) or into the optic chiasm (5-10 nl), as predicted by stereotaxic coordinates. MR images were acquired at 11.7T (Bruker BioSpin MRI Inc.) using a 20mm RF birdcage coil. Slab images of the optic tract (0.45cm thick slab with 32 slices) were acquired at 6 minutes intervals for 2 hours beginning 30 minutes post injection with a 3D RARE sequence (TR/TE 300/5 ms, 4 echoes, 1 averages, FOV 1.5^2x0.45 cm, and 128^2x32 matrix size). Whole brain images were acquired at 24 h after $MnCl_2$ injection with a T_1 weighted 3D UFLARE sequence (TR/TE 300/5 ms, 4 echoes, 2 averages, FOV 2.2x1.5^2 cm, and 256x128^2 matrix size).

Results and Discussion

VEPs of a sighted mouse (Fig. 1) before (top), 3 hours (middle) and 24 hours (bottom) after injection of $MnCl_2$ demonstrate a transient loss of neuronal electrical activity in response to light after $MnCl_2$ injection. Both eyes are affected, although only one was injected. MRI

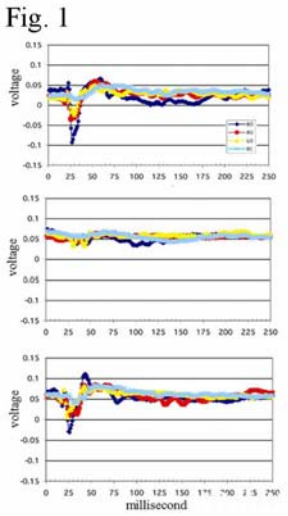

Fig. 1

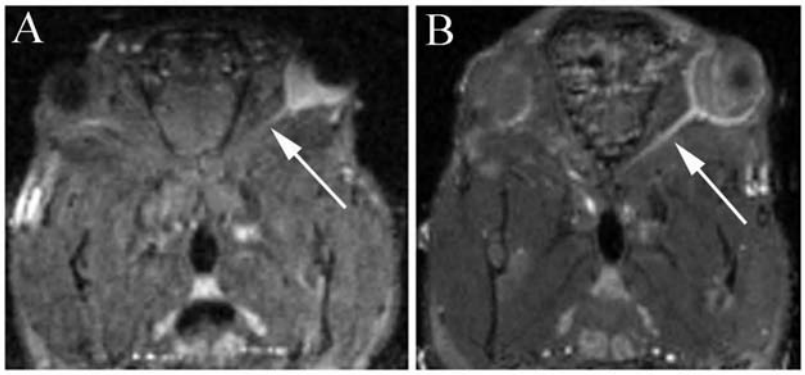

Fig. 2

Fig 1: VEPs measured before (top), 3h after (mid) and 24h after $MnCl_2$ occular injection. (BO:both eyes open, LO: left open, RO: right open, and BC: both closed). Fig. 2: (A) Slab MR image at 30m post injection, (B) 3-D MR at 24h.

at 30 minutes after injection displays a hyper-intense signal in the vitreous with some increased intensity in adjacent portion of the optic nerve (Fig. 2A). By 24 hours after injection, hyperintense signal appears throughout the optic nerve (Fig. 2B), as well as in the colliculus and sometimes was detectible in the visual cortex. Images captured at 6 minute intervals after injection reveal that anterograde transport is slow. Preliminary measurements suggest ~5 µm/min, analogous to the rate of mitochondrial transport. In contrast, transport from the chiasm was uniquely retrograde at early time points and rates varied widely between mice. Transport rates were similar in sighted and blind mice.

Conclusion

Visual response does not appear to affect Mn^{++} transport in the optic system. Mn^{++} injection into the eye disrupts the light response, but the eye recovers within 24 hr at low Mn^{++} doses. The rate of anterograde transport from the eye and unidirectional retrograde transport from the chiasm demonstrate that (1) Mn^{2+} transport is not via diffusion but involves an active transport mechanism; (2) Mn^{2+} may re-distribute by either anterograde or retrograde transport, depending on the site of uptake.

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2. Pautler, R. G., Mongeau, R. and Jacobs, R. E. (2003). "In vivo trans-synaptic tract tracing from the murine striatum and amygdala utilizing manganese enhanced MRI (MEMRI)." Magn Reson in Med **50**(1): 33-39.