MRI Tracing of Manganese Transport in the Mouse Brain After 3rd Ventricle Application

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The elucidation of neural connectivity is essential for understanding development, function, and plasticity. Manganese-enhanced MRI (1, 2) allows *in vivo* neuronal tract tracing. In this study $MnCl_2$ was injected into the mouse brain third ventricle and the spatiotemporal distribution of Mn^{2+} enhancement assayed *in vivo* high resolution MRI. Brain structures highlighted by Mn^{2+} accumulation were compared with high resolution *ex vivo* MR images and histological atlas data.

Materials and methods

Male mice (C57BL/6, age 10 weeks) were anesthetized with ketamine and xylazine i.p. and positioned in a stereotaxic frame. The site of injection in the third ventricle was determined as 2.5 mm posterior to bregma and 3 mm below the pial surface based on mouse brain stereotaxic coordinates. A burr hole was drilled and 20 nL of 200 mM $MnCl_2$ was injected via pulled glass capillary and then the incision was closed with tissue adhesive.

MR images were acquired on a DRX500 spectrometer with microimaging accessory (Bruker BioSpin MRI Inc.) using a 20 mm birdcage coil. Scans were acquired 30 min, 8 hr and 24 h after $MnCl_2$ injection with a 3D UFLARE sequence (TR/TE 300/5 ms, 4 echoes, 2 averages, FOV 2.2x1.5x1.5 cm, and 256x128x128 matrix size). Vital signs were then monitored continuously and isoflurane anesthesia was adjusted to maintain breathing rate at a normal level (150-160/min).

After the final imaging time point, the brains were fixed with 4% paraformaldehye (PFA) via cardiac perfusion, the head was move and maintained in 4% PFA for 4 hours. Samples were then immersed in 20 mM Prohance and 0.01 % sodium azide for ten days. For imaging, specimens immersed in Fomblin and data acquired using a 3D spin echo protocol (TR/TE 50/6 ms, 8 averages, FOV 25x12.5x12.5 mm and 512x256x256 matrix size), yielding an isotropic spatial resolution of $(49\mu m)^3$.

Results and Discussion

At 30 minutes after the injection of $MnCl_2$ into the third ventricle application, the ventricles appear hypointense due to T_2 shortening by high local Mn^{2+} concentration, while diffuse hyperintensity is seen in periventricular regions and spatially distinct enhancements are seen in the choroid plexus, hippocampal, and medial hypothalamic areas (fig. 1). Short time signal changes within and directly adjacent to the ventricles likely result from the direct diffusion of Mn^{2+} in the CSF and through the ventricle ependymal walls into adjacent parenchyma. Short and long time specific enhancement of selected regions, on the other hand, is probably caused by axonal transport of the Mn2+ taken up by neuronal cells. Both the 30 minute and 8 hour scans show the detail structure of hippocampus from the dentate gryus to the CA1 subfield (Fig. 1). At 24 hours after Mn^{2+} injection (Fig. 2) enhancement is predominantly observed in GABA-ergic rich regions: CA3 field of hippocampus, substantia nigra, thalamus, and hypothalamus; suggesting the anterograde axonal transport of Mn^{2+} in GABA-ergic fibers.

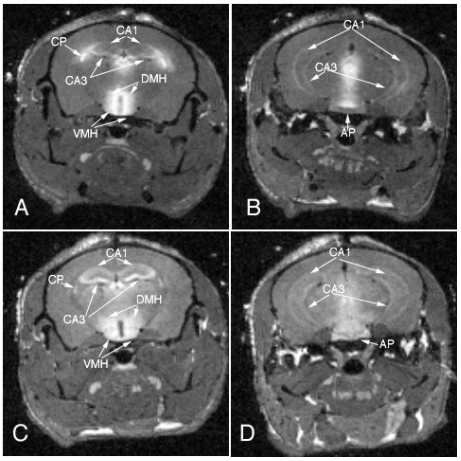

Figure 1. 3D UFLARE MRI of the moues brain 30 minutes (A&B) and 8 hours (C&D) after third ventricle injection on $MnCl_2$. The images in A and B show peri-ventricular enhancement. C and D show enhancement in the hippocampus and medial hypothalamic region. CP, choroid plexus; DMH, dorsomedial hypothalamic nucleus; VMH, ventromedial hypothalamic nucleus; AP, anterior pituitary.

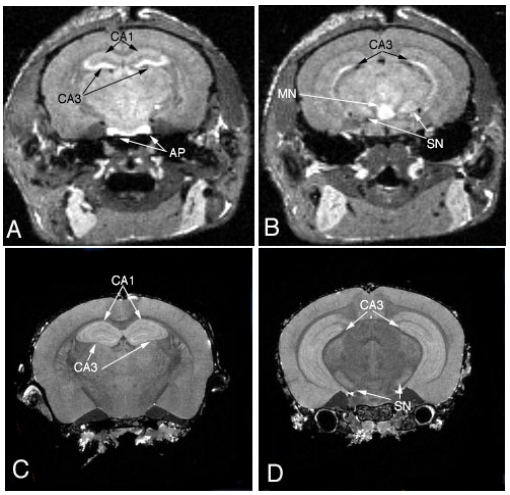

Figure 2. 3D UFLARE MRI of the brain 24 hours (A&B) after the third ventricle injection and corresponding findings in 3D T1 weighted MRI of fixed brain (C&D). Note the pronounced enhancements in the CA3 and nucleus, and week enhancement in the CA1 subfield. AP, anterior pituitary; SN, substantia nigra; MN, mammillary nucleus.

Conclusions

In this work we test the feasibility of administering Mn^{2+} into the third ventricle in an attempt to achieve a relatively uniform initial spread of Mn^{2+} in the mouse brain. In short times after administration diffusion distributed the ions throughout the ventricular system and into parts of the brain within several millimeters of the ventricles. At long times after administration, specifically enhanced regions were observed in parts of the brain far removed from the ventricles, indicating transport via neuronal activity. This methodology should prove useful in analyzing neuronal connectivity in mutant and transgenic mice.

References 1, Pautler RG, Mongeau R, Jacobs RE. Magn Reson Med. 2003,50:33-39.

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