Manganese Neurotoxicity in MEMRI Optic Tract Visualization.

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Introduction:

Manganese (Mn^{2+}) is a paramagnetic calcium analogue that can enter neurons through calcium channels, and is transported along microtubules via fast axonal transport. Manganese enhanced MRI (MEMRI) neuronal tract tracing has produced much excitement in the last few years as a relatively non invasive, in-vivo method of directly visualizing neuronal pathways. However, Mn^{2+} is known to be a neurotoxin and this aspect of MEMRI has not yet been scrutinized thoroughly. We performed a series of experiments to address the biological effects of using Mn^{2+} as an MRI contrast agent at the concentrations reported as used in the literature.

Methods:

34 NIH Swiss mice were used in these studies. Animals received a 1μl injection of Mn^{2+} into the anterior eye chamber under Ketamine/Xylazine anesthesia. Imaging was performed at 18 hours post injection using Isoflurane anesthesia. The mice were imaged in vivo with a 1.5cm custom built surface coil and scanned in a horizontal bore 7T MR scanner (Varian) using a T_1 weighted 3D FLASH sequence TR/TE= 28/6.45, FA 25˚, FOV 18mm, 256^3 matrix (60 μm isotropic resolution). Images were analyzed using intensity maps for anatomical areas or enhancement, volume measurements and 3D segmentation/rendering of optic tracts.

Experiment 1: To measure the effect of concentration of injected Mn^{2+}, Mn^{2+} solution was injected at 4 different concentrations: 1M, .5M, .25M, and .125M. The intensities of the MR signal was measured at 6 set points along the optic tract in each brain (vitreous, optic nerve, chiasm, lateral geniculate body, superior colliculus, V1 visual cortex). **Experiment 2:** Repeated injections were made into the eye and intensities measured as before to determine if the capacity for neuronal transport of Mn^{2+} had been affected by the first study. **Experiment 3:** Tissue intensities were measured at multiple time-points after the injection of 1M solution to monitor the dispersion of MR visible Mn^{2+} from the brain tissues.

Results:

Experiment 1: At all the 4 concentrations used for this experiment the visual tract was clearly outlined from eye to superior colliculus (figure 1). Signal intensity increased with increasing concentrations of Mn^{2+} except at the highest concentration (1M, n=30) where intensity in the structures beyond the eye was lower than the 0.5M concentration injection (figure 2). **Experiment 2:** In animals that had had 2 Mn^{2+} injections, the signal intensity in the brain structures after the second injection was reduced by 50% compared with after first injections (figure 4). **Experiment 3:** By day 6 the tissue signal intensity had reduced in intensity by 90% of that on day 1 and had reduced by a further 5% by day 13 (figure 3). A further concerning observation was the lens could be seen to opacify as the Mn^{2+} was being injected. Also in 80% of the mice who had the 1M Mn^{2+} injections there where physical changes apparent in the eye.

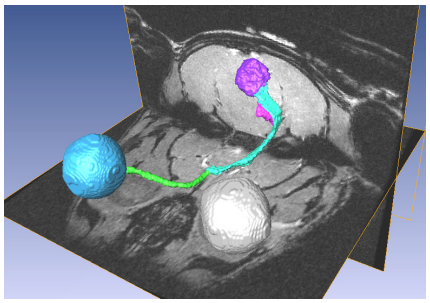

Figure 1.

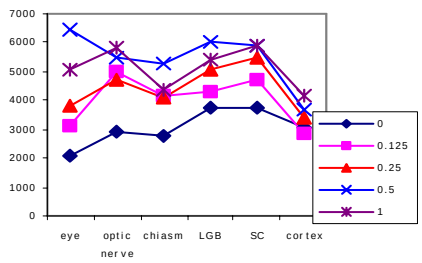

Figure 2.

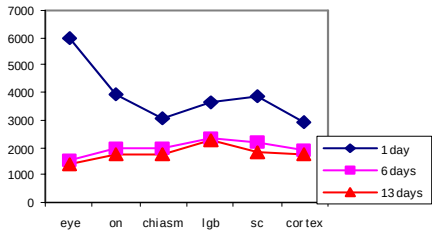

Figure 3.

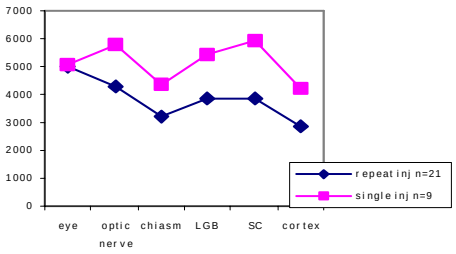

Figure 4.

Discussion and Conclusion.

The reduced signal intensity in the brain structures of the animals that had had 2 Mn^{2+} injections, and the reduced intensity in the animals who had had a 1M injection compared to lower doses both suggest that intra-ocular injection of Mn^{2+} at this dose is neurotoxic enough to potentially damage the white matter tracts that they are outlining. The optic tract was adequately outlined at all doses used and it may be possible to further reduce the dose given. The Mn^{2+} injection at lower doses may be safer, though the optimal concentration for a 'safe' dose, if any, needs to be established if this technique is to be used for the longitudinal monitoring of degenerative brain diseases.