MRI manganese enhanced neuronal tract tracing: a spatial statistical evaluation in the mouse.

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INTRODUCTION

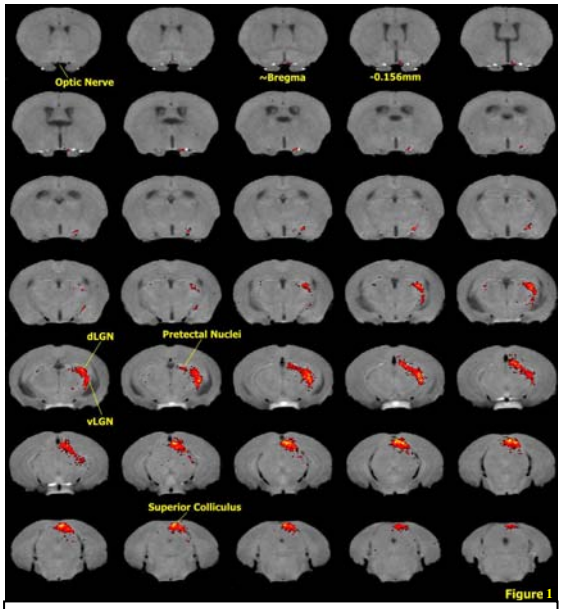
Manganese (Mn) enhanced magnetic resonance imaging (MRI) is a relatively new technique with considerable potential applications. The sensitivity and specificity in examinations of anatomy or function will ultimately depend on the pharmacology of Mn. At concentrations sufficient to visualise using MRI, Mn behaves as a calcium analogue, entering neurons via calcium-signalling machinery at a rate that is dependent on activity; being subsequently transported through efferent tracts. Our previous work [1] revealed neural tract tracing is facilitated by an optimal concentration load, with higher concentrations apparently reducing Mn transport. At an optimal source concentration of Mn within the intra-vitreal compartment, the extent of visible Mn tract tracing in adult mice was assessed. To enhance sensitivity, high resolution 3D anatomical images were normalised to a standard mouse template. This study details a quantitative group statistical parametric evaluation of Mn enhanced MRI anatomical tracts, specifically designed to reveal second order visual thalamo-cortical projections.

METHODS

Conducted under licence from the UK Home Office. **Intra-ocular injections:** Approximately 24 hours prior to MR imaging, adult female C57/BL6 mice (21±2g) were anaesthetised and a pulled glass capillary inserted into the intra-ocular space at the temporal retinal margin. Manganese chloride (25mM/ml), dissolved in physiological saline, was injected to a volume of 0.5µL (n=6). **3D MR Imaging:** Under urethane anaesthesia (i.p. 2g/kg), animals were 3D spoiled gradient echo imaged: acquisition matrix 512x128x200; TR=35ms; TE=4ms; nex=4; α=21°; scan time=60minutes. **Post-processing:** Zero-filling providing an effective image resolution of 78x78x78µm. Images were B1 corrected [2] and normalised. Gaussian smoothing for second analysis: 156µm FWHM kernel. **Analysis:** A general linear model [3], characterising positive changes in contrast between naïve controls (n=8) and Mn enhanced subjects (n=6) at each individual voxel, determined group statistical parametric maps (SPM) (uncorrected threshold of $p<0.001$ – based on a priori anatomical hypotheses with corrections applied at the cluster level [4]). SPM's are overlaid on mean images derived from all 14 animals.

RESULTS

MR imaging of manganese loaded vitreous fluid has demonstrated differential spatial changes in contrast consistent with manganese transport within primary retinal efferents. Group statistical analysis between naïve and manganese injected animals demonstrated robust statistically significant clusters (corrected at the cluster level $p<0.001$) within the primary retino-tectal, retino-thalamic and accessory systems - figure 1. Gaussian smoothing the images improved sensitivity sufficiently to resolve the ipsi-lateral island within the dorsal lateral geniculate nucleus (dLGN) - figure 2. No regions associated with second order projections of the visual pathways were noted in either analysis.



Group Statistical Parametric Map illustrating the spatial extent of statistically significant contrast between naïve controls (n=8) and animals injected 24 hours prior with manganese (n=6). Images are presented as interpolated slices of 156µm thickness. Colour scale: T distributions equal to or greater than statistical significance (T>3.9, uncorrected threshold $p < 0.001$).

Repeat analysis with the data prior smoothed with a Gaussian kernel of 156µm. Images are presented 78µm thick slices. Colour scale: T distributions equal to or greater than statistical significance (T>3.9, uncorrected threshold $p < 0.001$).

DISCUSSION AND CONCLUSION

The SPM demonstrated robust statistically significant clusters limited to terminal fields of first order retinal ganglion neurons. Since manganese loading within the vitreal space was optimised [1], increasing the intra-vitreal loading concentration would only reduce transportation to the primary terminal fields. Thus this study suggests that the technique of manganese enhanced tract tracing may only be suitable for the visualisation of first order neuronal tracts and their respective terminal fields in the mouse. However, as <3% of retinal ganglion cells project ipsi-laterally in the pigmented mouse [5], the demonstration of the ipsi-lateral dLGN island (the first reported by Mn enhanced MRI) and only an approximately in-plane size of 100x200µm, demonstrates the degree of sensitivity afforded by spatial group statistical mappings.

REFERENCES

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