Quantitative Manganese Tract Tracing: Concentration Dependence

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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 using Mn will ultimately depend on a thorough knowledge of its pharmacology. At concentrations sufficient to visualise using MRI, Mn behaves as a calcium analogue and so enters neurons at an activity dependent rate via voltage gated channels. Studies in calcium metabolism, chronic Mn toxicology and synaptic neuro-transmission suggest an agonistic then antagonistic effect on calcium signalling machinery with increasing concentration load. Given such concentration dependent properties together with the activity dependent entry into, and anterograde transport[1] within, the cell, the efficacy of manganese tract tracing may critically depend on initial and sustained activity at the site of Mn loading. This study details a quantitative evaluation of Mn enhanced retino-tectal projections in the adult mouse, reporting optimal, unfavourable and adverse consequences of differential intra-ocular loading.

METHODS

This study was conducted under licence from the United Kingdom Home Office. All MRI measurements were performed on a 7T (Magnex Scientific, Abingdon, UK) super-conducting, horizontal bore magnet. ***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-vitreal space at the temporal retinal margin. Different concentrations of manganese chloride, dissolved in physiological saline (6.25, 12.5, 25, 50, 100, 400 and 800 mM/ml), were injected to a volume of 0.5μL (n=3, except 800mM/ml solution – n=1) ***3D MR Imaging*****:** Under urethane anaesthesia (i.p. 2g/kg), animals were 3D spoiled gradient echo imaged with different applied RF flip angles of α_2=21º (acquired first) and α_1=8º: acquisition matrix 512x128x200; TR=35ms; TE=4ms; nex=4; scan time=60minutes. Images were zero-filled providing an effective image resolution of 78x78x78μm. A ratio method algorithm determined T1 relaxation times on a pixel-wise basis[2]. Maximal contrast images were used to contour the ipsilateral and contralateral superior colliculus (SC) and intra-ocular volumes. SC regions of interest were transposed onto the T1 maps and mean T1 relaxation times determined. A mean reference T1 measure was determined for each animal, the forepaw representation (S1) within the somatosensory cortex, and each animal's mean SC T1 relaxation time normalised to the reference T1.

RESULTS

Varying concentrations loaded into the vitreous humor demonstrated differential changes in T1 relaxation times in the ipsi- and contra-lateral SC (fig 1): univariate analysis of variance demonstrated a main effect of concentration ($F_{(6,28)}$=5.9, p<0.001). Intra-ocular volumes delineated by residual manganese enhancement were determined (fig 2). A one-way ANOVA determined a main effect of concentration on measured intra-ocular volumes ($F_{(7,5)}$=5.192, p=0.026). Post-hoc Tukey tests revealed statistically significant differences between various concentration loads and the 400mM load (fig 2). The 800mM loading concentration demonstrated terminal labelling presumably due to a dilution effect associated with the substantial increase in intra-ocular volume - effective intra-ocular concentration < 400mM load.

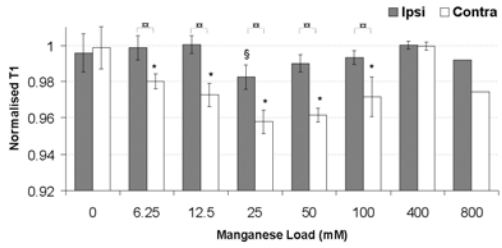

Figure 1: Average T1 relaxation rates for contoured superior colliculi. (*) between naïve and 400mM contral-lateral T1 times ($p \leq 0.041$); (§) between ipsilateral T1 times in naïve, 12.5mM and 400mM subjects ($p \leq 0.018$); (¤) between ipsilateral and contralateral T1 times at each concentration load ($p \leq 0.032$).

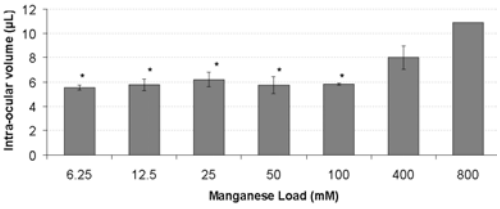

Figure 2: Contoured ipsi-lateral intra-ocular volumes at varying concentrations of manganese. (*) illustrate statistically significant differences from the intra-ocular volume after 400mM of manganese ($p \leq 0.011$).

DISCUSSION AND CONCLUSION

Varying manganese concentration load within the vitreous humor of adult female mice has confirmed differential degrees of resultant tectal terminal labelling. The statistically significant decreases in T1 relaxation times within the contralateral superior colliculus are consistent with the bulk of retino-tectal projections decussating at the optic chiasm. Quantitative evaluation of T1 relaxation times within the SC has enabled the robust assessment of the within and between subject effects of intra-ocular manganese loading. The differential decreases in T1 relaxation times within the SC suggest varying concentrations of manganese may result in optimal, unfavourable and undesirable consequences. The applied load of 25mM manganese yielded maximal changes in T1 relaxation times within both SC, while the 400mM dose appeared to completely block T1 changes in all terminal fields and significantly increased intra-ocular volume. Given such a marked change in intra-ocular volume, the 800mM manganese load was considered undesirable. The previous Mn enhanced tract tracing studies have utilised manganese concentrations in the range of 200-800mM. Consequently, the current study has implications for the design of optimal future studies.

REFERENCES

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