Signal Enhancement Achieved Through Hypotonic Bath Application of Manganese Chloride in MR Microscopy of the Rat Hippocampal Brain Slice Model

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INTRODUCTION

The employment of manganese ion (Mn^{2+}) as a contrast agent in magnetic resonance imaging studies has continued despite its know toxicity due to its abilities to offer signal enhancement in T1-weighted images, to act as a retrograde tracer capable of delineating neuronal pathways, and to track calcium-dependent biological processes with MRI. Manganese deposition into neural tissues can be achieved by systemic administration through intravascular infusion [1], or IP/SC injection [2] but requires relatively large doses of manganese-containing compounds. Direct stereotactic microinjection into specific brain regions is possible, but results in a localized concentration gradient as manganese ions diffuse away from the injection site [3]. In the present study, live hippocampal slices were extracted and immersed in baths containing variable concentrations of manganese chloride. Although manganese deposition was not uniform in all slices tested, it was sufficient enough to elicit a nearly five-fold (4.82X) signal increase while preserving or enhancing the contrast between hippocampal layers.

METHODS

Excised rat hippocampi from adult Sprague Dawley rats were cut into 500µm thick slices using a McIlwain tissue chopper and immersed in solutions of manganese chloride tetra-hydrate (10µM, 20µM, 30µM, 40µM, 60µM, 80µM, or 100µM) in ddH_2O. Control slices were prepared as above and immersed ddH_2O containing no manganese. Following a 1h incubation, hippocampal slices were loaded into a multislice chamber [4] and imaged in a 600MHz (Oxford Instruments) or 750MHz (Bruker) spectrometer. Our imaging protocol consisted of standard, T1-weighted (TR/TE = 500ms/5.3 ms), multislice (thickness = 0.25 mm), spin-echo images taken with an in-plane resolution of 150µm. Signal to noise ratios were calculated for each slice and expressed as a percent increase from the untreated slice and grouped (n = 5) according to treatment condition before undergoing ANOVA statistical analysis. In slices with unequal uptake of manganese ion, ROI's were taken where the concentration of manganese was greatest. Following analysis of variance, a post hoc test that employed the Bonferroni correction to adjust for multiple comparisons was used to determine if significant differences were present between groups.

RESULTS

All groups save for that exposed to the lowest concentration of manganese (10µM) achieved statistically significant (α = 0.05) signal increases as compared to controls. Also, between-group comparisons which attained statistically significant differences were the 40, 60, and 80 µM groups as compared to the 10µM group (Fig. 1). Groups exposed to concentrations above 100µM exhibited dramatic signal loss due to the T2-shortening effects of manganese (Fig. 2). The signal enhancing effects present at lower concentrations was retained in tissues for up to 24 hours following the initial exposure (Fig. 2). Lastly, the non-native contrast elicited by certain levels of manganese uptake results in clear delineation of structures such as the stratum lacunosum and stratum lucidum suggesting reduced uptake of manganese ion as compared to adjacent tissues (Fig. 3).

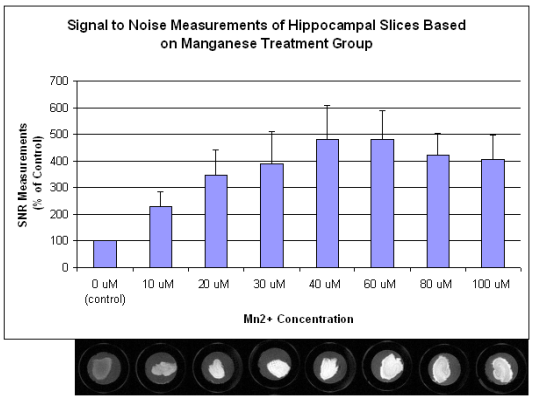

Figure 1. Mean signal to noise ratios for all Mn2+ treatment groups (n = 5) are reported as % of control. All treatment groups (save 10µM) showed significant signal increases (α = 0.05). The 40, 60, and 80 µM treatment groups were also significantly increased as compared to the 10µM group. Inset MRIs show representative images from each group.

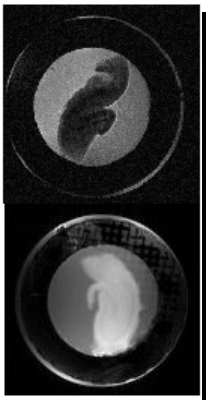

Figure 2. Top: Hippocampal slice exposed to 1000µM Mn2+. Bottom: Slice from 40µM treatment group imaged 24h after treatment.

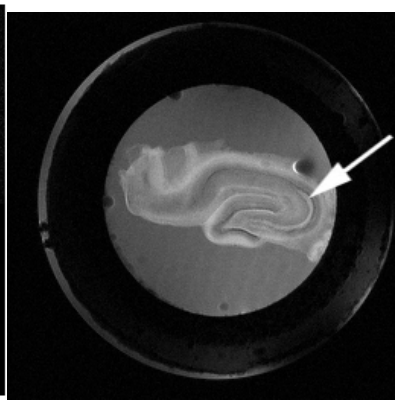

Figure 3. Hippocampal slice from the 100µM treatment group. The laminar anatomy is revealed in stark, non-native contrast. Structures such as the stratum lucidum (arrow) appear brighter due to T2-shortening effects in the surrounding tissue. Res. = 39µm in-plane.

DISCUSSION and CONCLUSIONS

Although manganese has been used, for example, as a contrast-enhancing device for liver imaging in humans [5], its future potential for clinical use depends on developing compounds and protocols which reduce its toxic effects. In this study, we have demonstrated a method for depositing manganese in the brain slice model and obtaining significant signal enhancement that may be traded for improved spatial resolution and/or imaging time. Further studies will optimize Mn concentration as a function of TR, and compare its effectiveness with contrast agents such as gadolinium (Gd). Since Gd complexes remain extracellular, and have thus been used to estimate extracellular volume fraction [6], they may be less effective than those which can enter both tissue compartments.

REFERENCES and ACKNOWLEDGEMENTS

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