

Manganese-enhanced MRI for phenotyping brain-wide activity in a mouse model of emotional learning and memory

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Introduction. Manganese-enhanced MRI (MEMRI) is a valuable tool for *in vivo* mapping of brain activity. The objective of this study was to use MEMRI for phenotyping differences in brain activity in mice selectively bred for behavioral alterations in fear learning and memory.

Methods. Mice (C57/B6 x DBA/J2 outbred strain) were selectively bred over 3 generations for a High (n = 7) and Low (n=7) Pavlovian fear conditioning behavioral phenotype. Manganese chloride (MnCl₂; 180 mg/kg total dose) was dissolved in a Bicine buffer (400 mM; 7.4 pH) and administered via subcutaneous osmotic pump implant (Model 1003D, 1 μl/hr flow rate, 3 day duration; Alzet, Durect Corp, Cupertino, CA USA). Control mice were not implanted with a MnCl₂ loaded pump (n = 2). Immediately following the end of MnCl₂ dosing, mice were fear conditioned (day 3 of MnCl₂ dosing) and immediately prepared for MRI scanning.

MRI protocol. All MR imaging was conducted using a 7 T Bruker BioSpec imaging system (Bruker NMR, Inc., Billerica, MA) with 20-cm horizontal inner bore diameter, superconducting magnet (Magnex Scientific, Abingdon UK), 80 mm quadrature transmit coil, and a dedicated phased array head coil with parallel imaging. Anesthesia was delivered using isoflurane (1.5 - 2%) and body temperature was maintained by a heating pad embedded in the animal holder. Animal temperature, respiration rate, and heart rate were monitored throughout all MRI scans. Images were acquired using a 3D RARE sequence with the following parameters: TR = 200 ms, TE = 6.8 ms, Rare factor = 2, 100 μm isotropic voxels, scan time = 1h 12 min. Additional images were acquired using a 3D FLASH sequence with the following parameters: TR = 50 ms, TE = 3.1, 100 μm isotropic voxels, scan time = 1 hr 12 min.

Data analysis. We used a 3D region of interest (ROI) tool for quantifying T1 weighted signal intensities for various brain regions (VivoQuant, Invicro, Boston MA USA). ROIs for the amygdala, central nucleus of the amygdala (CeA) and the CA3 pyramidal cell layer were hand drawn. To minimize intersubject differences unrelated to MnCl₂ concentration, the ROIs of each subject in the Mn+ and control group were normalized to the subjects own cortex ROI signal intensity. Next, percentage change in signal intensity was calculated for each subject in the Mn+ group relative to control. The dependent variable for all statistical analysis of between-group variations in MnCl₂ concentration was percentage change. All multiple comparisons (ROIs) were analyzed first using multivariate statistics followed by one-way ANOVAs, when necessary. Statistical significance was set at *p* < 0.05.

Results. Overall, the signal intensity for the RARE MRI sequence was greater (5% change) compared to the FLASH MRI sequence (0.24% change; *p* = .003; Figure A). Enhancement in the signal intensity for the RARE compared to FLASH image sequence was found for all ROIs analyzed, except the medulla, pallidum, olfactory bulb, amygdala and CA3 pyramidal cell layer (Figure A, C and D). These data indicate region-specific T1 weighted signal enhancement using RARE compared with FLASH MRI sequences. Next, we compared signal intensity differences for both RARE and FLASH image sequences between phenotype (HIGH x LOW) and across all ROIs (multivariate analysis). Results showed no significant differences in the signal intensity between phenotype for the FLASH sequence. However, results showed a statistically significant enhancement in the signal intensity for the hypothalamus (*p* = .01), striatum (*p* = .04) and pallidum (*p* = .02) in the LOW compared to HIGH phenotype (Figure B). Signal intensity differences did not correlate with fear conditioning behavior for any of the ROI measured.

Discussion. Here we quantitatively showed enhanced MEMRI signal intensity using RARE compared to FLASH imaging sequence. However, signal enhancement was not uniform across all brain regions analyzed. This finding suggests that different imaging sequences may be used to achieve optimal contrast, especially for MEMRI experiments with multiple ROIs. Phenotypic differences were found for the hypothalamus, striatum and pallidum. Due to the ability of Mn²⁺ to enter neurons primarily via voltage-gated Ca²⁺ channels, these data suggest phenotype-dependent changes in the functioning of these brain regions. Because changes in the signal intensity for these regions were not correlated with fear conditioning, we can conclude that they represent non-specific brain functioning likely related to home-cage behavior. This finding is of particular interest because these regions are thought to be components of a neural system centrally involved in mood disorders (Price and Drevets, 2010). Further, we did not find a correlation between the signal intensity for any other brain region and fear conditioning behavior (the amygdala and/or hippocampus were hypothesized to show a change). We believe this lack of correlation is due to an incongruent time course of Mn²⁺ uptake (osmotic pump is slow) and behavior (fast). For future work, we intend to develop a protocol for delivering Mn²⁺ into the brain at a temporal resolution that matches functional brain activation underlying fear learning and memory.

