Mouse Brain Structure and Metabolic Stability Follows Focused Beam Microwave Irradiation

M. D. Boska[1], E. McIntyre[1], M. L. Mellon[1], and H. E. Gendelman[2]
[1]Radiology, University of Nebraska Medical Center, Omaha, NE, United States, [2]Pharmacology and Experimental Neurosciences, University of Nebraska Medical Center, Omaha, NE, United States

Introduction Focused beam microwave irradiation (FBMI) uses a high power microwave pulse for rapidly heating brain tissue. This serves to partially denature proteins and halt enzyme activity. FBMI is a superior method to rapid freezing for preserving in vivo brain metabolism levels for analysis. Previous works using magnetic resonance spectroscopy (MRS) have focused on exploiting this property for determining protein phosphorylation and rat brain metabolic content. The current study investigated the stability of MR imaging metrics (including T_1, T_2 and DTI metrics) and ^{1}H MRS of mouse brain over intervals of hours after two levels of irradiation. This allowed FBMI to be optimized for high resolution morphometric or spectroscopic studies of mouse brain.

Materials and Methods: ***FBMI*** For the in situ studies, 10 male NOD-scid-IL-2R gamma chain null – (NSG) mice were sacrificed by FBMI using a 10 kW Muromachi Microwave Fixation System (Stoelting Co, Wood Dale, IL, USA) using methods approved by the University of Nebraska Medical Animal Care and Use Committee. Microwave power was set to 4 kW for 0.7 or 0.9 s. ***MRI/MRS***. MRI/MRS was performed using a 7 T small animal scanner (Bruker Biospin, Billerica, MA). In-vivo data were acquired using volume coil transmit, surface coil receive. In vitro data were acquired using a home built solenoid transmit/receive coil. After euthanasia, heads were excised, skin and eyes removed and the sample was placed in a container of Fomblin for susceptibility matching. The chamber was evacuated to remove air bubbles. T_1 measurement was performed using a fast spin echo with 18 slices, TRs from 0.18 to 14 s (Fig 1A). T_2 was measured using a CPMG phase cycled spin echo with 28 slices, 10 echoes, from 11 to 110 ms (Fig 1B). For both measurements: slices in coronal direction, slice thickness = 0.5 mm contiguous, in-plane resolution 128 x 128, 20 mm FOV. For DTI, 12 diffusion directions, *b*-value = 800 s/mm^2, 7 averages using a single shot EPI acquisition (Fig 1C,D). Localized ^{1}H MRS were acquired on a 4 mm^3 voxel centered in the mouse brain using PRESS with TE = 33ms, TR = 4s, 256 averages (Figure 2). Data were acquired in-vivo, followed by microwave euthanasia. Subsequently, all measures were acquired once every 1.14 hours for a total of 16 hours following FBMI. ***Spectroscopic Analysis***. Spectra were analyzed using the QUEST algorithm in the jMRUI package (Figure 2). The basis set spectra were generated using GAMMA and validated using phantoms. Basis set included alanine, ascorbate, aspartate, choline, creatine, GABA, glucose (alpha and beta), glutamate, glutamine, glutathione, GPC, glycine, lactate, myo-inostitol, NAA, NAAG, phosphoethanolamine, phosphorylcholine, scyllo-inostitol, and taurine. Single acquisition water spectra were acquired for concentration reference. Concentrations (institutional units) are not corrected for T_1 or T_2 of either water or metabolites.

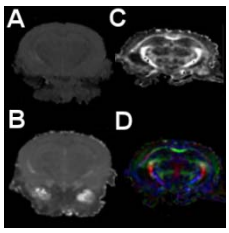

Figure 1. *Maps of A: T_1, B: T_2, C: fractional anisotropy and D: color encoded primary eigenvalues of DTI.*

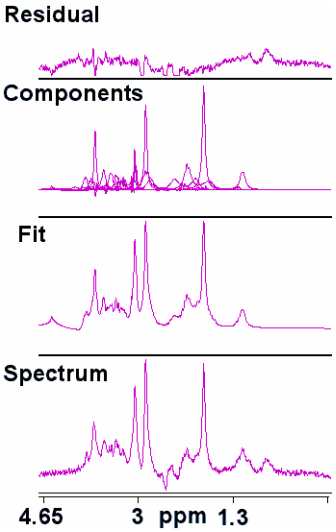

Figure 2. *Example of an in-vivo spectrum acquired prior to FBMI and associated fit.*

Results: ***Hippocampus Relaxation Times***. Water T_2 was found to be unaltered by FBMI at either irradiation time. However, T_1 was reduced at the higher power, while T_1 remained at in-vivo levels for the duration of the measurement (Fig 3A). Measures from other regions of interest show similar trends. ***Hippocampus DTI.*** FA was found to be unaltered at shorter FBMI time, but was reduced at longer FBMI time (Fig 3B). Mean diffusivity was reduced at both times due to temperature change, but was reduced to a slightly greater degree by longer FBMI time. ***Central brain metabolite levels.*** Levels of NAA+NAAG (and all other metabolites) increase immediately after irradiation with longer FBMI, but NAA+NAAG is reduced at shorter FBMI (Fig 3C). Over the course of the 16 hours, slight decreases in NAA+NAAG and slight increases in lactate are seen (Fig 3D). It may be possible to further reduce this rate of NAA reduction and lactate accumulation by slight increases in FBMI time. The shorter FBMI time shows immediate NAA loss and lactate accumulation. Other interesting trends include progressive increases in taurine and alanine over time after irradiation, especially after short FBMI time.

Discussion. Trends from the two levels of irradiation tested suggest that lower time, while not providing complete deactivation of enzymatic activity, maintains better structural integrity than longer irradiation. Conversely, for spectroscopic studies, longer FBMI time provides a window of several hours with no significant degradation of metabolic concentrations over time. Increased concentrations observed upon irradiation may be due to changes in metabolite relaxivity, loss of water, change in ventricle contribution to water reference, or increased visibility with cessation of enzymatic activity. FMBI should prove useful for extending spectroscopic and quantitative structural acquisitions to higher resolution and higher dimensionality as well as aiding coregistration to brain histology.

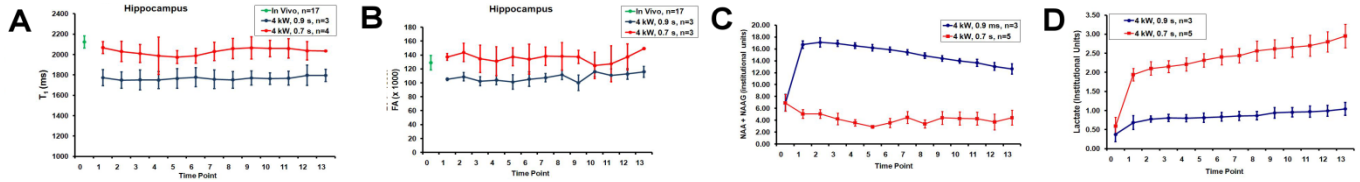

Figure 3. *Results from 0.7 ms FBMI (red) and 0.9 s FBMI (blue). Time points are in intervals of 1.17 hours as stated in Materials and Methods. Time 0 represents the in-vivo measures prior to FBMI. A. T1 of the hippocampus shown in Figure 1. The green point is the result of the in-vivo measurement prior to FBMI. B: Fractional anisotropy, C: NAA concentration and D: Lactate concentration.*