

**Purpose:** Develop focal stereotactic proton irradiation for accurate spatial localization (< 2mm) in the rodent brain. Planning and evaluation was performed using Micro-Computed Tomography (CT) and magnetic resonance spectroscopy (MRS).

**Method and Materials:** Rodents were visualized using Micro-CT; the data was transferred to Optirad (proton treatment planning system) for planning the site of brain irradiation (20 Gy). After irradiation the animals were positioned for proton magnetic resonance spectroscopy (MRS) on a 4.7T imager. Acquisition conditions for metabolite assessment allowed complete relaxation between excitations. A 5 mm<sup>3</sup> voxel was placed at the irradiation site. Care was taken to avoid bone-tissue interfaces. The MRS data was collected into 2048 points (frequency resolution of 1.2 Hz/point), a relaxation delay of 4s and an echo time of 120ms. A water presuppression pulse reduced the water peak below 1%. The MRS acquisition parameters were; a field of view of 5 cm, two acquisitions, a matrix size of 256 X 128 for a total imaging time of 8.5min.

**Results:** At 12 hrs after focal irradiation, MRS revealed the presence of a lactate (Lac) peak that did not resolve during the course of the 5-day imaging series. In addition, the peak height of N-acetyl-aspartate (NAA) was reduced at 12 hrs compared to choline (Cho) and creatine (Cre) peaks. The NAA changes relative to Cho/Cre appeared to resolve over the 5 day time course.

**Conclusion:** Focal stereotactic proton irradiation (<2mm) can be obtained when combined with CT and MRS. Validation of high dose focal irradiation by MRS suggested cellular injury as evidenced by a persistent lactate peak, indicative that cellular necrosis continues for at least five days . Transient neuronal injury was shown by reduced NAA/(Cho+Cre) at 12h with a partial recovery over the next five days. Histology was performed to further validate these findings.