Tracking of Neuroprogenitor Cells in Association with Traumatic Brain Injury

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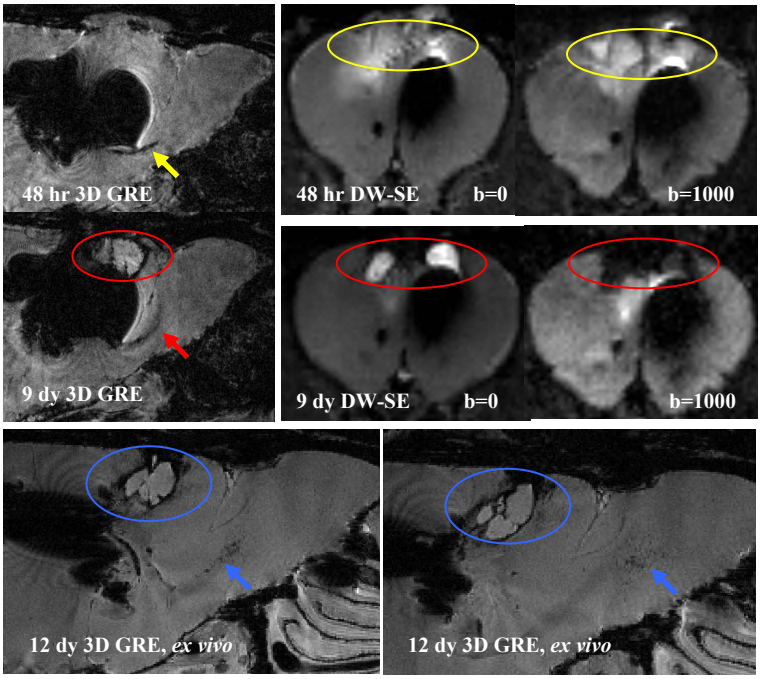
Introduction: Neuronal progenitor cells (NPC) from the subventricular zone (SVZ) are known to migrate continuously along the rostral migratory stream (RMS) to the olfactory bulb (OB). Research suggests that these cells also can migrate to lesions outside the RMS. This study aims to investigate if the migration of endogenously labeled NPCs will be altered, either by reduced RMS migration or re-routing to the site of traumatic brain injury (TBI), following a controlled cortical impact (CCI).

Background: Neuroprogenitor cells reside in the subventricular zone, which is located next to the lateral wall of the lateral ventricle. NPCs are capable of endocytosing magnetic micron-sized particles and can be tracked *in vivo* along the RMS using MRI [1]. In the SVZ, these cells are primarily B type cells with properties similar to that of astrocytes [2]. They divide and later form neurons during migration along the RMS toward the OB. Recent studies have suggested that NPCs residing in the SVZ also participate in remodeling after brain injury and are responsible for the astrocyte scar following TBI. These labeled cells therefore should be trackable in association with brain injury such as CCI [3].



Methods: Four week-old Sprague-Dawley (SD) rats were anesthetized and put in a stereotactic frame. The CCI procedure involved a 5-mm craniotomy immediately anterior to Bregma followed by a piston impact induced at a speed of 2.0 m/s and 2 mm deep. To label proliferating cells in the SVZ, a 50-μL solution of 0.86-μm encapsulated, fluorescent magnetic beads (Bangs Laboratories, Fisher, IN) was injected 2 mm rostral, 2 mm lateral (right) from Bregma and 3 mm deep. The animal was imaged at 48 hrs and 9 days post surgery at 21.1 T with a diffusion-weighted spin echo (DW-SE) sequence with b = 0 and 1000 s/mm^2 and 3D T_2*-weighted gradient echo (GRE) sequence (TE/TR= 5/50 ms, 50x50x200 μm resolution). At day 12, the animal was sacrificed and perfused for an *ex vivo* high resolution GRE scan (60-μm isotropic resolution).

Results: Images on the left show GRE and diffusion weighted images from 48 hrs and 9 days post surgery. At 48 hours, hypointensities (yellow arrow) are seen at the beginning of the RMS with the GRE images. In the DW-SE scans, it is possible to visualize the CCI in the form of hyperintense signal (yellow ellipse) resulting from inflammation and restricted water diffusion. The injection of the magnetic beads is also seen as a large void that obscures the ventricles. At 9 days, the TBI has developed into a necrotic core (red ellipse) mostly consisting of freely diffusing water while NPCs have migrated farther along the RMS (red arrow), although the full extent of this movement and the RMS are somewhat obscured by susceptibility distortions induced by the magnetic particles in the ventricles. At this time point, it is possible to visualize a rim of dark contrast around the TBI, which results partially from the migration of cells from the SVZ as corroborated by fluorescence imaging (not shown). The phenotype of these cells is currently under investigation using immunohistochemistry.

Two different partitions of 3D high resolution dataset acquired from the same animal sacrificed 12 days post surgery are also shown above. Labeled cells (blue arrows) along the RMS are clearly evident as hypointense voxels. These signal voids are likely NPCs and their progeny as they migrate into the RMS and eventually the OB. The necrotic cores of the CCI (blue ellipses) are seen above the ventricles and again show signs of dark contrast in the periphery of the lesion, which partially represent cells migrating from SVZ to the CCI as part of either inflammatory response or regeneration in the lesion penumbra.

Discussion: This preliminary study shows that it is possible to label NPC in association with neuronal damage such as TBI and track NPC migration along the RMS and towards the induced lesion. The more anterior site of magnetic bead injection may have been beneficial for accessing the SVZ by initially disrupting the cell layer. This effect may have sped up the labeling process because the initial labeling and migration time course in this effort appears to have been more rapid than previously reported. Although NPCs continue to migrate along the RMS, at least a portion of SVZ cells appear to have been re-directed to the site of CCI. Further investigation is underway to confirm the origin of labeled cells in the lesion periphery and identify the status and type of those cells.

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