COMBINED IN VIVO MR AND OPTICAL IMAGING OF STROKE INDUCED NEUROGENESIS IN THE MOUSE BRAIN.

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Background: Neurogenesis in the adult brain occurs in the sub-ventricular zone (SVZ), and new cells migrate along the rostral migratory stream (RMS) to become mature interneurons in the olfactory bulb. This phenomenon is upregulated following stroke and may contribute to brain repair (1-4). Neurogenesis is typically studied using invasive techniques, such as injection of the thymidine analogue bromodeoxyuridine (BrdU). Recently, transgenic mice were generated that express Discoma sp reef coral red fluorescent protein (DsRed) (5), or the bioluminescent enzyme luciferase (LUC) (6), in response to activation of the migrating neuroblast promoter doublecortin (DCX). The aims of this project are to establish a multi-modal, combined magnetic resonance imaging (MRI) and optical imaging protocol to monitor the timecourse and expression patterns of the neurogenic response *in vivo* prior to and following focal ischemia in these mice.

Methods:
Animal model: Male CD1 DCX-LUC mice (37-44g) (n=4) and C57/BL6 DCX-DsRed mice (29-35g) (n=3) received different durations (30, 20, or 10min) of transient middle cerebral artery occlusion (MCAO); one C57/BL6 DCX-DsRed mouse received a sham procedure.
Imaging: MRI was performed 4 days prior to, and 7 days post-MCAO, on an 11.7T Biospec (Bruker BioSpin) equipped with gradient coils of 740mT/m, 72mm quadrature resonator, and mouse quadrature surface coil. The scan package was composed of a spin echo T_2-weighted scan (TR/TE: 4000/10msec, 16 echoes, FOV: 12.8 x 12.8mm^2, matrix: 128 x 128, 16 contiguous slices 0.5mm thick) and a fast low angle shot (FLASH) 3D scan (TR/TE: 100/3.8msec, FOV: 12.8 x 12.8 x 12.8mm^3, matrix: 96 x 96 x 96). Optical imaging was performed 8 days prior to, and 12 days post-MCAO, in a photon imager (Biospace) to detect fluorescence (C57/BL6 DCX-DsRed) or bioluminescence (CD1 DCX-LUC) following intraperitoneal administration of 150mg/kg of the substrate luciferin.
Histology: Two DsRed mice (including the sham) were sacrificed at 7 days post-MCAO and the other animals at 12 days. Mice were perfusion fixed and tissue processed for immunohistochemistry for various cell lineage markers in combination with reporter expression.

Results: Ischemia durations of 30min produced extensive lesions (Figure 1) and mortality, whereas smaller subcortical insults, with relatively little morbidity, were produced using a 10min occlusion period.

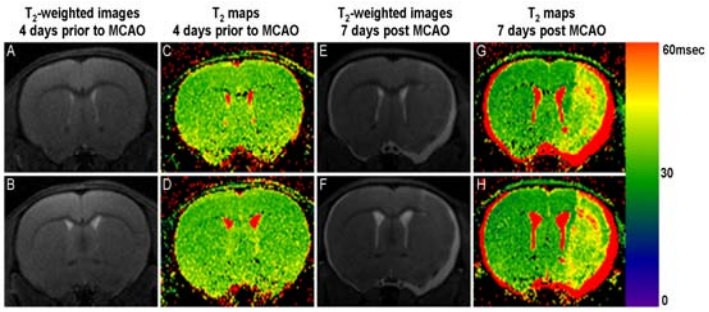

Figure 1. A-D: T_2-weighted images (10.2msec), and corresponding T_2 maps (msec), of coronal slices (0.4mm and 0.02 from bregma) from a CD1 DCX-LUC mouse 4 days prior to MCAO, and 7 days post-MCAO (E-F).

Prior to MCAO, photons produced from the oxidation of luciferin in the CD1 DCX-LUC mice were counted. Emission was highest in the head in a pattern that mimicked the pathway of the RMS in the brain (Figure 2C-D). The exact location is currently under investigation and will be determined using co-registration of the optical images to the FLASH 3D MRI data set (Figure 2A-B). Following MCAO the observed bioluminescent signal appeared to broaden and expand into the injured hemisphere (Figure 2E-F). No fluorescent signal was detected from the C57/BL6 DCX-DsRed animals *in vivo*.

Conclusions: These promising results indicate that monitoring the neurogenic response *in vivo* following stroke is possible using these transgenic animals. In addition to the optimization of a multi-modal imaging protocol, we are currently also establishing a timecourse of LUC expression in these animals following MCAO.

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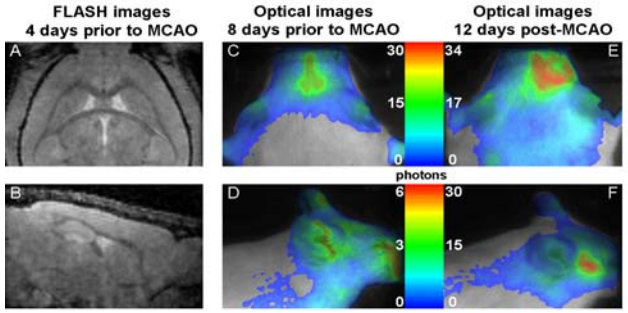

Figure 2 A-B: Horizontal and sagital FLASH images from a CD1 DCX-LUC mouse 4 days prior to MCAO. C-D: Photon count images from the same animal at 8 days prior to, and at 12 days post-MCAO (E-F).

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