In vivo detection of cFos-GFP for neural activation via Magnetization Transfer Contrast (MTC) Magnetic Resonance Imaging (MRI)

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Introduction: There has been great interest in non-invasive imaging not only for diagnosis but also for tracking disease progression. Imaging is necessary at the pre-clinical level to better investigate responses to novel treatment as well as early diagnosis that can then be translated into the clinic. Green Fluorescent Protein (GFP) is a widely used molecular and gene expression marker. However, its use in *in vivo* imaging has been limited to transparent tissue due to the technical limitations of optical imaging. We have previously described a novel approach to detect Green Fluorescent Protein (GFP) expression in vivo using Magnetization Transfer MRI[1]. Here, we apply the MRI based detection of GFP to evaluate a cFos-GFP fusion model[2]. cFos is a widely utilized marker of neuronal activation that currently requires *ex vivo* immunohistochemistry for detection. We believe detecting cFos expression *in vivo* with MRI would provide an unparalleled means to assess neuronal activation in mouse models of human diseases.

Methods: **Animal experiments:** Transgenic cFos-GFP fusion mice[2] were obtained from Mutant Mouse Regional Resource Centers (Chapel Hill, NC). The strain is kept as a hemizygous which allowed us to use wildtype littermates as controls. We induced cFos activation through osmotic stimulation as previously described[2]. Specifically, mice were injected with a hypertonic solution (2 M NaCl; 10µl/g body weight) intra-peritoneally to induce dehydration. All the animals used in this study were handled in compliance with institutional and national regulations and policies. The protocols were approved by the Institutional Animal Subjects Committee at Baylor College of Medicine.

Imaging Protocol: Animals were anesthetized by isoflurane gas at a 5% in oxygen and placed into a mouse holder where they were kept under anesthesia at a nominal 2% isoflurane in oxygen. Imaging was performed utilizing a Bruker Avance Biospec, 9.4 T spectrometer, 21 cm bore horizontal imaging system (Bruker Biospin, Billerica, MA) with a 35 mm volume resonator. During imaging the animal body temperature was maintained at 37.0°C using an animal heating system (SA Instruments, Stony Brook, NY). Animals were imaged as quickly as possible after injection of the hypertonic solution as a baseline. Then the same scan was performed 2 hours post injection. MT imaging was performed with a novel 3 Dimensional (3D) protocol where saturation is applied per 3D phase step. Scans were run under a Fast Steady State Precesion (FISP) protocol on FID mode with a TE of 1.5 ms and a scan repetition time of 500 ms. The excitation pulse was Gaussian with a flip angle of 40. Saturation was achieved as previously reported[1] with a square pulse with B_1 = 12 µT, frequency offset = 1kHz, pulse length = 40 ms, and number of pulses = 36 for a total saturation time of 1.44 s. For all scans the geometry and location was kept constant with Field of view = 20 mm isotropic and matrix = 128 isotropic for a resolution of 0.156 µm isotropic. Two scans were performed per data point, each with 4 averages: without (~4 min) and with saturation (~16 min) for a total time of ~20 minutes per data point.

Data Analysis: Magnetization Transfer Ratios (MTR) in the form of *MTR = (Unsaturated - Saturated) / Unsaturated* were calculated. Pixel by pixel MTR calculations were performed in MATLAB (The Mathworks, Natick MA) to generate pseudo-colored images. Overlay images were prepared in Adobe Photoshop by setting the lower values of the MTR map to be transparent.

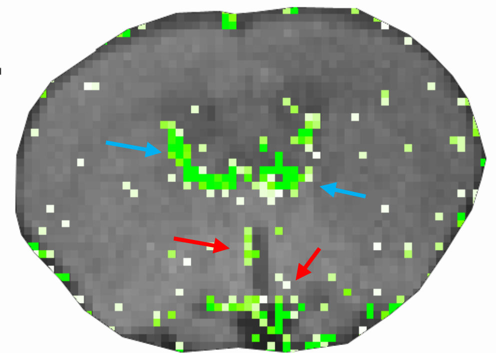

Figure 1. Example of an MTR brain map overlay (short axis view) from a cFos-GFP mouse exposed to a dehydration protocol normalized to control. We collected 3D MTC datasets before the dehydration protocol and also 2 hours after dehydration for one control and one cFos-GFP mouse. The bright areas correspond with increased MTR in cFos-GFP mice in comparison to control. In this case, we observed an increase in the hypothalamic PVN (red arrows) similar to what was previously reported[2] and also the thalamus (blue arrows)

Results: The normalized results are depicted in Figure 1. Normalization was achieved first by subtracting the baseline MTR for both groups and then by subtracting the control from the cFos-GFP. Imaging results for the animals were 3-dimesionally aligned prior to the last subtraction for ease of analysis. We observed significant increases in the MTR in the paraventricular nucleus (PVN) which is consistent with what has been reported in the *in vitro* characterization of this model. However, we also observed MTR increases in the thalamus.

Discussion: Our goal is to develop a platform to directly asses neural activity with a cFos-GFP/MTC imaging strategy. Our *in vivo* preliminary data are consistent with what has been previously reported *in vitro* in the cfos-GFP mice in response to osmotic stimulation. This approach could potentially allow the possibility of non-invasively assessing treatment response and monitoring disease progression over time that is applicable to a myriad of neurological diseases. While fMRI is already in use to evaluate neuronal activation in the clinic, it is an indirect measurement based upon changes in blood flow. fMRI is also very difficult to perform in mouse models due to their small size. Detecting cFos expression *in vivo* with MRI would allow for an unprecedented understanding of neurodegenerative disease processes.

Acknowledgements: We would like to thank the Mouse Imaging Centre (MICe) of the Hospital for Sick Children in Toronto for providing us the *MICe-build-model* package to perform the image registration.

References: 1. Perez-Torres CJ, et al. *Neuroimage Submitted* 2.Barth, AL, et al. *J. Neurosci.* **24**, 6466-6475 (2004).