Opto-fmri in awake rodents: activation and deactivation fMRI signals induced by excitation and inhibition of neurons

L. Becerra[1,2], G. Brenner[2,3], J. Bishop[1], P-C. Chang[1], H-S. Shin[3], A. Yang[4], M. Baratta[4], P. Monahan[4], E. Boyden[4,5], and D. Borsook[1,2]

[1]A. Martinos Center, Massachusetts General Hospital, Boston, MA, United States, [2]Harvard Medical School, Boston, MA, United States, [3]Anesthesiology and Critical Care, Massachusetts General Hospital, Boston, MA, United States, [4]Media Laboratory, Massachusetts Institute of Technology, Cambridge, MA, United States, [5]Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, United States

Introduction: Functional MRI has been used extensively to determine brain function in a variety of conditions in humans and other species. Excitation of neurons has been correlated with activation detected by fMRI. Deactivation in fMRI, however, has not been clearly established although it has been associated with neuronal inhibition. Here, using opto-genetic techniques, neurons in rat cingulate cortex were transfected to express ArchaeRhodopsin (ArchT) channels that inhibit neuronal firing under illumination (Chow et al. 2010). In addition, neurons in somatosensory cortex were transfected to express ChannelRhodopsin-2 (ChR2) channels that excite neurons when illuminated (Han et al. 2009). Upon optical stimulation of somatosensory cortex, positive activation in the transfected area was detected and found to be proportional to the light power. When the cingulate cortex was optically stimulated, a negative (deactivation) response was observed, also proportional to the light power level. Inhibition of the cingulate resulted in activation in other areas of the brain. Histological studies indicate that only neurons are transfected, hence, these results suggest that neuronal excitation produces fMRI activation and that inhibition results in fMRI deactivation.

Methods: Animals: Male Sprague-Dawley rats (~300 g, N=6) were prepared with 2 cannulas; one positioned in the anterior cingulate cortex (ACC) and one in the somatosensory cortex (S1) (face region). The cannulas were utilized for virus delivery as well as fiber optic positioning for light stimulation. ACC was transfected to express (ArchT) and S1 ChR2. Imaging took place 3 weeks after infection. fMRI was carried out on a 9.4T Bruker scanner. A RARE sequence was used to capture anatomical images (0.5 mm slices (24), 0.23x0.23 voxels. An EPI sequence was used to acquire functional data (Twenty 1 mm thick slices, 0.47 mm in plane resolution, TR/TE=3s/10ms). Rodents were awake during the experiment (Becerra et al. 2010). ArchT transfected neurons are most sensitive to green light (532 nm) while ChR2 are to blue (473 nm). Three power levels were used for each laser (color): 3, 10, and 30 mW. The paradigm consisted of 30 seconds rest and 21 seconds illumination, repeated 5 times.
Analysis: Data was analyzed with fsl tools (www.fmrib.ox.ac.uk/fsl) adapted for rodents (Becerra et al. 2010). Statistical maps were overlapped with anatomical scans.

Results: ArchT (Inhibition): Negative (deactivation) is observed in response to stimulation of transfected neurons in ACC (Figure 1 upper row). Deactivation strength (percent signal change) as well as volume is directly proportional to the level of illumination power. The Figure depicts deactivation clusters thresholded at the same level (z=-5); pink cluster (10 mW) is smaller in size than the blue one (30 mW). At this threshold, no significant deactivation is observed with 3 mW light power. ChR2 (Excitation): Positive activation is observed around the area of S1 illumination. Activation strength and volume is directly proportional to the level of illumination power (Figure 1 lower row).

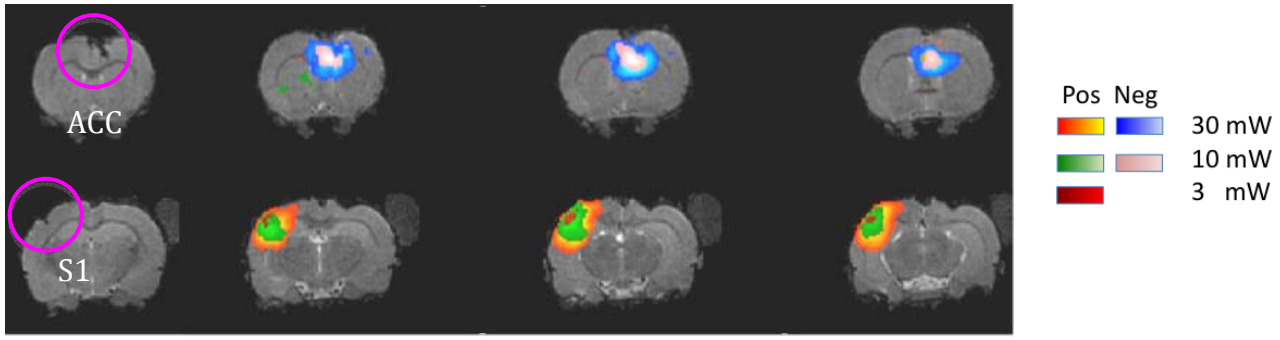

Figure 1: Transfected neurons in ACC with ArchT (Top Row) produce a deactivation BOLD response when illuminated. The size and strength of the deactivation is proportional to the intensity of illumination. Transfected neurons in S1 with ChR2 (Bottom Row) produce a positive BOLD response when illuminated. Similarly, size and strength of activation is proportional to illumination intensity.

Discussion and Conclusion: Activation and deactivation was observed in brain structures transfected for either excitation or inhibition of neurons under illumination, respectively. The observed changes are not due to thermal effects because they are opposite in valence (positive and negative activation) and experiments on uninfected, but cannulated, rats, did not induce any BOLD changes. Optical stimulation only drives transfected cells, neurons in this case, suggesting that excitation of neurons induces a positive BOLD signal while inhibition produces a negative one.

References: Becerra et al. NeuroImage Sep 9 (2010), Chow et al. Nature, 7;463(7277):98-102 (2010). Han et al., Neuron 62(2):191-8 (2009).