Impact of Anesthesia on Optogenetically Activated Medical Prefrontal Functional Network in Rats

Zhifeng Liang[1,2], Glenn D.R. Waston[2,3], Kevin D. Alloway[2,3], Gangchea Lee[1], Thomas Neuberger[1], and Nanyin Zhang[1,2]

[1]*Dept. of Biomedical Engineering, Pennsylvania State University, University Park, PA, United States,* [2]*Center for Neural Engineering, The Huck Institutes of Life Sciences, Pennsylvania State University, University Park, PA, United States,* [3]*Neural and Behavioral Sciences, College of Medicine, Pennsylvania State University, Hershey, PA, United States*

Introduction: It is widely recognized that anesthesia has profound impacts on almost all aspects of brain functions. Specifically, we have previously demonstrated the impact of anesthesia on global organization[1] and local circuits[2,3] in rodents using resting-state functional MRI (rs-fMRI). To further explore this impact in task based fMRI, we utilized the technique of optogenetics-fMRI (opto-fMRI[4,5]) to examine the global impact of optically induced neural activation in cognition related high order cortex of infralimbic cortex (IL, part of medial prefrontal cortex) in rodent.

Methods: Seven adult male Long-Evans rats underwent a surgery for injecting viral vectors of channelrhodopsin (ChR2) (AAV1.CamKIIa.hChR2(H134R)) and implanting optical fiber (0.4mm diameter) in IL. After 2-3 weeks of recovery, animals were acclimated to MRI environment as previously described[6,7], and then imaged in the awake or anesthetized (1-1.5% isoflurane) condition in separate sessions on a 7T Varian system. Blue pulses were delivered through a patch cable connected with the ferrule with the parameters: 10 Hz for 15 or 30 s, or 20 Hz for 1 s, inter stimulus interval 30s, estimated light power at fiber tip 15mw. For the MRI experiment, anatomical images were first acquired with the fsems sequence, followed by the acquisition of gradient-echo EPI images covering the whole brain (TR = 1 s; TE = 16.8 ms; matrix size = 64×64; FOV = 3.2×3.2 cm; slice number = 20; slice thickness = 1 mm). Anatomical images were manually aligned to a segmented rat brain atlas. EPI images were motion corrected, and spatially smoothed (FWHM 1mm). Motion parameters were regressed out from the time course of each voxel, and voxel time courses were linearly detrended. A Fourier analysis paradigm was adopted from a previous opto-fMRI study [4]. Briefly, for each individual voxel, the time series was Fourier transformed to the frequency domain, and the coherence (c) value was calculated as follows: $c = \frac{|F(f_0)|}{\sqrt{\sum_f |F(f)|^2}}$ where

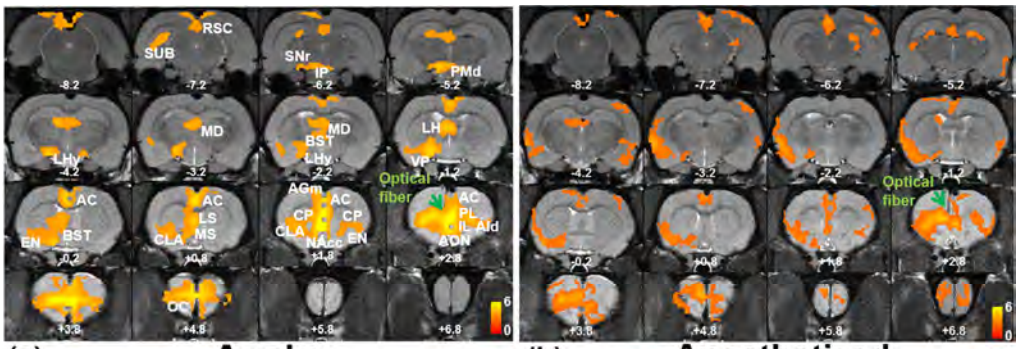

Fig. 1. Activation maps in response to IL optogenetic stimulation in awake and anesthetized rats. a, the averaged activation map at the awake state. b, the averaged activation map at the anesthetized state. Green arrow, implanted optical fiber. Distance to Bregma is labeled in each slice. Both maps were displayed at the threshold of z > mean+2SD ($p<0.05$ at the whole brain level). Distance to Bregma is labeled in each slice. AC, anterior cingulate cortex. PL, prelimbic cortex. AON, anterior olfactory nucleus. AId, dorsal agranular insular cortex. AGm, medial agranular (prefrontal) cortex. CP, caudate putamen. CLA, claustrum. NAcc, nucleus accumbens. EN, endopiriform nucleus. LS, lateral septal nucleus. MS, medial septum. BST, bed nucleus of stria terminalis. LHy, lateral hypothalamic area. MD, mediodorsal nucleus of thalamus.

$|F(f_0)|$ is magnitude of the stimulation frequency component, and $\sqrt{\sum_f |F(f)|^2}$ is the sum of squares of magnitudes of all frequency components. Coherence values were further converted to z values. To threshold the z images, we adopted an adaptive thresholding strategy[8]. The distribution of z values is assumed as a mixture of a Normal distribution and a Gamma distribution, with z values of true activation follow the Gamma distribution and noises follow the Normal distribution. Thus, all z values from mean z images were fitted by a Normal-Gamma mixture model defined as follows: $p(z) = c * N(z|\mu,\sigma) + (1-c) * G(z-\mu|\alpha,\beta)$ where *N(.)* and *G(.)* Normal and Gamma distribution. *c*, *μ,* and σ^2 are the proportion, mean, and variance of Normal distribution, respectively. *α* and β are two parameters of Gamma distribution. Z value threshold was set based on the estimated mean and variance of Normal distribution of a given condition. For all rats, injection sites and ChR2 expression were verified with histology.

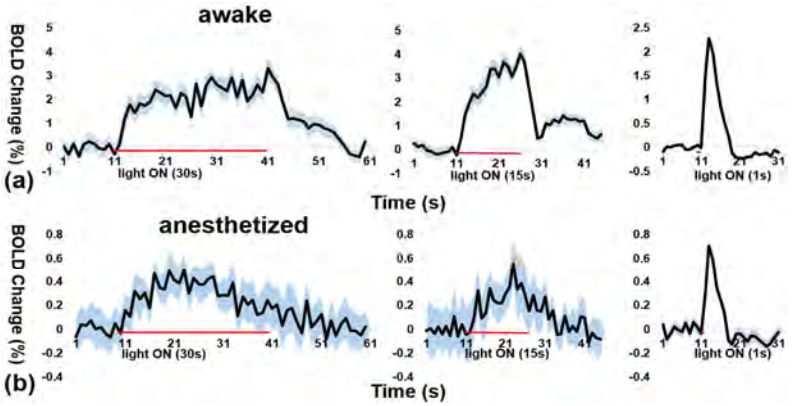

Fig. 2. Averaged time courses of optogenetic stimulation in awake (a) and anesthetized (b) conditions in anterior cingulate cortex (AC). x axis, time (s). y axis, percentage BOLD signal change. Red lines indicate stimulation periods. Blue shades in standard error of mean(SEM).

Results and discussion: Optogenetic stimulation in IL generated robust local and distal BOLD signal activation across the brain in both awake and anesthetized states (Fig. 1). However, the spatial extent of the activation was reduced in the anesthetized state, particularly in regions like anterior cingulate cortex, lateral and medial septum. Moreover, the amplitude of BOLD activation greatly diminished (Fig. 2) with all three stimulation periods (30, 15 and 1s). In summary, this study indicated that anesthesia greatly reduced the BOLD activation of cognition-related functional network. In addition, opto-fMRI provides a unique approach to investigate high-order cognitive systems in animal studies.

Acknowledgement: R01MH098003 and R01NS085200 to Nanyin Zhang.

References: 1. Liang, Z., King, J. & Zhang, N. *The Journal of neuroscience* 32, 10183-10191(2012). 2.Liang, Z., King, J. & Zhang, N. *NeuroImage* 59, 1190-1199 (2012). 3. Liang, Z., Liu, X. & Zhang, N. *NeuroImage*, doi:10.1016/j.neuroimage.2014.10.013 (2014). 4. Lee, J. H. *et al. Nature* 465, 788-792 (2010). 5. Desai, M. *et al. Journal of neurophysiology* 105, 1393-1405 (2011). 6. Zhang, N. *et al. Journal of neuroscience methods* 189, 186-196 (2010). 7. Liang, Z., King, J. & Zhang, N. *The Journal of neuroscience* 31, 3776-3783 (2011). 8. Allen, E. A. *et al. Frontiers in systems neuroscience* 5, 2, (2011).