Probing Gq-GPCR Signaling in Rat Primary Motor Cortex with Pharmacogenetic fMRI

Manasmita Das[1], Heather K Decot[1], Yu-Chieh Kao[1], Oyarzabal Esteban[1], and Yen-Yu Ian Shih[1]

[1]*Biomedical Research Imaging Center, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States*

TARGET AUDIENCE fMRI, molecular imaging, and systems neuroscientists.

INTRODUCTION. In recent years, the advent of novel genetic tools has triggered a renaissance in neuroscience research, providing tremendous opportunities to study the neuronal correlates of complex brain function with high spatiotemporal control. Among these, optogenetics[1,2] and pharmacogenetics[3,4] have emerged as two powerful experimental modalities that allow exquisite manipulation of neural circuits through selective expression of 1) light sensitive membrane channels (opsins) and 2) synthetic G-protein coupled receptors (GPCRs) called **D**esigner **R**eceptor **E**xclusively **A**ctivated for **D**esigner **D**rugs (DREADDs). The latter prototype i.e. DREADDs utilize extrinsic muscarinic receptors (hM3Dq for excitation and hM4Di for inhibition) that have lost their affinity for endogenous acetylcholine but can be selectively activated by a synthetic and otherwise pharmacologically inert ligand, clozapine-N-oxide (CNO). Although signaling through GPCRs is slower relative to ion channels, DREADDs have more long-lasting effect and can target a much broader brain area. The major strength of DREADDs is their intrinsic sensitivity to an orally available and injectable agonist, which allows them to selectively and noninvasively target major GPCR signaling pathways (Gq, Gs and Gi) without the need of implanting any optical device. While optogenetic approach has received phenomenal attention of fMRI scientists[1], the feasibility of probing selective cellular or circuit activity using pharmacogenetic fMRI has never been systematically explored. In this study, we offer the first, direct evidence of fMRI responses to pharmacogenetic activation of excitatory neurons in rat primary motor cortex (M1) using Gq-coupled DREADDs. This novel technology contributes a potent toolbox for noninvasive mapping of GPCR-mediated cell signaling *in vivo*, a critical insight of which is necessary to understand various physiological and molecular processes and broaden the range of new therapeutic interventions.

METHODS. Male Sparague Dawley (SD) rats (250-300g) were microinjected directly into M1 region of the brain with an adeno-associated virus encoded by hM3Dq under Ca^{2+} /calmodulin-dependent protein kinase II promoter (CaMKII-α) and fused with the fluorphore m-Cherry. fMRI experiments were performed after 3 weeks of virus injection. Each rat was endotracheally intubated and ventilated with ~1.5% isoflurane and medical air. The ventilation rate and volume were adjusted to maintain end-tidal CO_2 (EtCO2) within a range of 2.6-3.2% and oxygen saturation (SpO2) above 96%. Rectal temperature was maintained at 37±0.5°C. During all imaging studies, animals were initially anesthetized using 1.5% isoflurane and then transferred to a well-established sedation protocol[5] using a combination of low-dose anesthetics (0.5% isoflurane) with intraperitoneally administered dexmedetomidine (0.1 mg/kg/hr) and pancuronium (1 mg/kg/hr). Cerebral blood volume (CBV)-weighted fMRI responses were measured using a steady state method[6] by intravenously injecting blood-pool type iron-oxide nanoparticles (30 mg/kg MION) through a tail-vein catheter. Single shot, single sampled GE-EPI sequences (BW= 300 kHz, TR= 1000 ms, TE= 8.107 ms, 80x80 matrix, FOV= 2.56 x 2.56 cm^2, slice thickness= 1 mm) were acquired using a Bruker 9.4T MR scanner and home-made surface coil. CBV data was continuously acquired for 30 mins and animals were intravenously injected with CNO (5 mg/kg) through the same tail vein catheter used for MION injection at 10 min after scan onset. Automatic co-registration using SPM codes were applied to realign time-series data within subjects and then again across subjects. Correlation coefficient (CC) analysis was used to generate fMRI map with reference to the pharmacognetic stimulation paradigm. ROIs were placed at the area showing virus expression as well as a control brain region in posterior parietal cortex. Animals were perfused immediately after fMRI experiments and virus expression in target regions was validated using confocal microscopy. Paired t-test was used to compare CNO-evoked CBV responses between virus-transfected and non-transfected sites. P <0.5 was considered as statistically significant.

RESULTS & DISCUSSION. Pharmacogenetic activation of excitatory neurons in rats transfected with AAV-hM3Dq-CaMKIIα-mCherry led to strong CNO-evoked CBV-fMRI responses in and around the site of virus infusion (**Fig. 1**). The local increase in CBV was significantly (P<0.05) higher compared to non-targeted, control brain areas in both hM3Dq transfected and sham subjects. Post-mortem histology revealed that hM3Dq transfected cells (labeled with m-Cherry) were confined to M1 region of the brain showing morphology identical to pyramidal neurons. These observations corroborate that selective activation of Gq signaling cascades in cortical neurons induce robust vasodilation, which plays an important role in modulation of functional hemodynamics. While CNO-induced CBV changes were more prominent in and around the site of virus expression, a careful inspection of datasets from our pilot studies revealed that CNO administration is associated with small fluctuations of baseline CBV (**Fig. 1**) even at nontrasfected control regions. This suggests the existence of some non-targeted interactions of CNO, which is thought to transiently affect animal physiology causing small changes in CBV. We believe that a careful optimization of CNO dose may help eliminating any nonselective pharmacological effect while improving the specificity of CNO-DREADD interaction

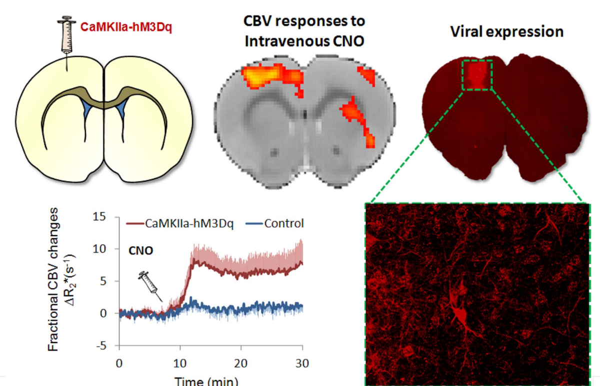

Fig 1. CBV-fMRI responses of rat brain expressing hM3Dq in the left M1 neurons following CNO injection. CNO-induced responses were only observed in the cortical areas with hM3Dq expression.

Conclusion and Future Directions. We have successfully demonstrated the feasibility of probing selective neuronal activity using a novel tool combining advantageous attributes of DREADDs and fMRI on the same platform. Our future study will exploit this newly developed technology to dissect the neuronal and astrocytic components of BOLD-fMRI signal and elucidate how neuronal and astrocytic GPCRs modulate functional hemodynamics *in vivo*.

REFERENCE [1] Lee et al., Nature 2010: 465, 788 [2] Figueiredo et al., Exp. Physiol. 2011: 96: 40. [3] Bryan et al. Mol. BioSystems 2010: 6: 1376 [4] Alexander et al. Neuron 2009: 63: 27 [5] Fukuda et al. Eur. J. Neurosci. 2013: 37: 80 [6] Lai et al. Neuroimage 2014: 84:11.