Targeting Projection Fibers for Optogenetics and fMRI

Xin Yu[1], Stephen Dodd[1], and Alan Koretsky[1]
[1]*NIH, Bethesda, MD, United States*

Target Audience Scientists studying animal models with fMRI, electrophysiology, and optogenetics will be interested in this work.

Purpose There is a growing interest in implementing of optogenetic tools in animal fMRI studie [1,2]. The optogenetic approach utilizes channelrhodopsins (ChRs) to elicit selective excitation of neurons. By expressing the light-sensitive opsin in neurons using cell-type selective promoters, specific neurons can be depolarized by light in a temporally precise manner with millisecond resolution. Recent reports have brought up two major issues of fMRI studies with *in vivo* optogenetics. One is that the light-driven neural activity while being cell specific may not correlate the fMRI signal with specific neural sources because fMRI detects integrated activity from local neural circuit [3]. The other issue is that direct photon delivery to targeted cortex can lead to heating artifact to confound fMRI signal [4]. To address these issues, an implanted optic fiber was used to target the axonal fibers of opsin-expressed neurons and fMRI signal was detected in cortical areas where axonal fibers project. This experimental design allowed us to control the projection activity in a fiber-specific manner and to detect reliable fMRI signal in cortex remote from the brain area being exposed to light. By targeting the ascending thalamocortical projection circuit, we detected light-driven fMRI activation in both primary (S1) and secondary (S2) somatosensory cortices. Furthermore, we targeted the opsin-expressed callosal fibers, which project to the deafferented barrel cortex in a rat model with unilateral infraorbital denervation (IO) [5]. fMRI detected not only the callosal mediated neural activity in the deafferented cortex by photon-stimulation, but also the back-propagated, antidromic activity in the contralateral barrel cortex of IO rats. Targeting projection fibers may be a useful approach to combine optogenetics and fMRI.

Methods BOLD fMRI studies were performed in Sprague-Dawley rats anesthetized with α-chloralose. Detailed IO surgical procedures and MRI scanner configuration (11.7T/31cm horizontal bore magnet) have been described previously [5]. BOLD functional map was acquired with 3D GE- EPI (TE 16ms, TR 1.5s, FA 12°, 300 micron isotropic). AAV viral vectors (*pAAV-CAG-hChR2-venus)* were injected into either ventral thalamus or the barrel cortex based on the brain atlas. Before fMRI scanning, an optic fiber (400μm) was implanted to target the callosal or ascending thalamocortical fibers to deliver 470nm blue laser light upon demand (5-20ms at 10-30 Hz). Light power was calibrated at the fiber tip when exposed in air (0.2-20mw). The exact photon density on the neural fibers can vary due to *in vivo* tissue/fiber tip environment. For block design experiment, 5-30s light on with 30s light off was repeated 5 times for each trial. 4-6 trials were required for each experiment. AFNI software was used for image analysis.

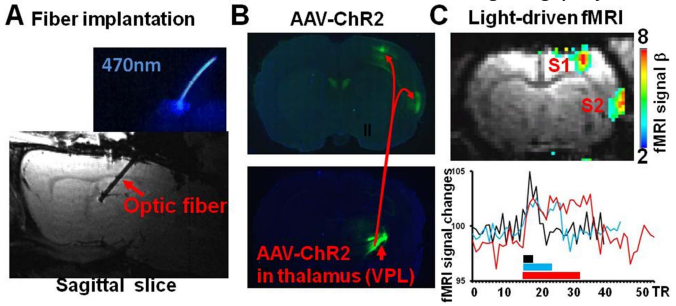

Fig 1. A. MRI images show optic fiber targeting thalamocortical ascending circuit (inset, 470nM blue light). **B.** Fluorescent images show the injection site of AAV-ChR-Venus to ventral thalamus (VPL) and the fluorescent signal in the projection cortical terminals at S1 and S2 cortical areas. **C.** BOLD fMRI images show light-driven functional maps (threshold at β=2) and the time course of fMRI signal with opton-stimulation at 5, 15 and 30s (lower panel).

Results The fMRI map from one rat with ChR2 expressed along the thalamocortical projections to both S1 and S2 areas is shown in Fig 1B. The location of light-driven fMRI signal coincides with the ChR-expressing fiber projection pattern detected in histology (Fig 1C). This result proved the feasibility to detect the fiber specific fMRI signal via optogenetics. Previously, we demonstrated that injury-induced plasticity leads to fMRI activation of the deafferented barrel cortex in response to the spared input from the same side whisker pad of IO rats [5]. To study if the callosal fiber mediates this plasticity, we used a fiber optic to target the opsin-expressing callosal fibers, which project to the deafferented cortex in IO rats (Fig 2 BC). Light-driven fMRI signal was detected in the deafferented barrel cortex of IO rats (Fig 2D, detectable at varied light power and duration in Fig 2E,F), but not in sham rats (data not shown). In addition, fMRI activation was detected in the contralateral barrel cortex, where opsin-expressing callosal projection neurons originate (Fig 2D). Light stimulation elicits the depolarization along callosal axons, which can back propagate to the soma to induce antidromic activity and lead to synaptic activity. This antidromic activity probably explains the fMRI signal in the contralateral barrel cortex (BC-I, Fig 2D).

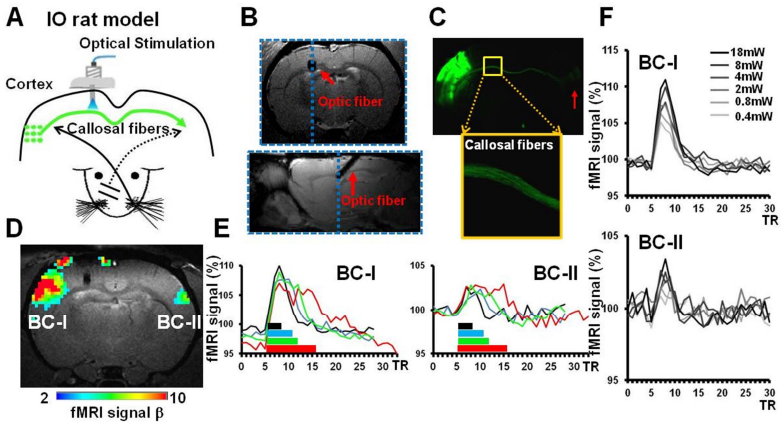

Fig 2. A. The diagram of optical stimulation of callosal fibers in IO rats. **B.** MRI images show optic fiber location. **C.** Fluorescent images show callosal fiber expressing ChR2 (arrow, callosal projection to the opposite barrel cortex, BC). **D.** fMRI images show light-driven activity in the BC of both hemispheres. **E.** fMRI time courses in BC-I and BC-II of IO rats at different duration of photon-stimulation (5,8,10,15s). **F.** fMRI signal at different light power (0.4-18mW) in BC-I and BC-II of IO rats.

Conclusion fMRI signals can be elicited using optogenetics targeted to activating specific neural fiber pathways. This is demonstrated for thalamocortical inputs and corticocortical inputs. A caution is that there is evidence for back propagation of neural signals leading to fMRI in retrograde directions. The combination of optogenetics and fMRI should enable detailed studies of the mechanism of synaptic plasticity.

Reference 1. Lee et al. *Nature* 465, 788-92, 2010. 2. Gerits et al. *Curr Biol* 22, 1722-6, 2012. 3. Logothetis, *Nature* 468, 2010. 4. Christie et al. *NeuroImage*, in press. 5. Yu et al., *Neuron*. 74, 731-42, 2012.