Frequency specific optogenetic recruitment of evoked responses in the somatosensory thalamocortical circuit

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TARGET AUDIENCE: Researchers who are interested in optogenetics and neural circuitry.

INTRODUCTION: Optogenetics with its ability of cell-specific, reversible and millisecond control of neurons and its demonstrated synergistic use with fMRI (ofMRI) [1] bridges the gap between the micro-scale (local microcircuits) and the macro-scale (brain regions) to infer large-scale functional connectivity in the brain. Despite the vast neuromodulation opportunity enabled by optogenetics, the effect of the specific stimulation frequency on the neural circuitry is not well explored. Hence, this study aims to elucidate the influence of optogenetic stimulation frequency on the dynamics of functional neural circuitry both spatially and temporally using fMRI. We chose to study the ascending somatosensory thalamocortical circuit by stimulating the ventral posterior nuclei (VP) due to a previous ofMRI studies with similar stimulation site [1]. We also stimulated a neighboring thalamic nuclei, the posterior complex (Po), to study the effect of stimulation frequency in a different subregion of the thalamus.

METHODS: Animal Preparation: Adult SD rats (~250g) underwent stereotaxic injection of AAV5-CaMKIIα::ChR2(H134R)-mCherry in VP (N=4, Fig. 1a) and Po (N=4, Fig. 2a). Four weeks after injection, a plastic fiber guide (480μm diameter) was implanted at the site of injection. **Optical Stimulation:** Light (473-nm wavelength, 30% duty cycle, 50mW/mm^2) was delivered at 10, 20 and 40Hz in an interleaved manner (Fig. 1b). Stimulation frequencies were chosen based on the range of frequencies used in previous ofMRI studies [1, 2]. **Data Acquisition:** fMRI data was acquired at 7T using single shot GE-EPI with TR/TE=1000/20ms, FOV=32x32mm^2, 64x64 matrix and 10 contiguous 1mm slices. High-resolution T2 images were acquired to show the site of stimulation in the thalamus. **Data Analysis:** GE-EPI images from each animal were realigned, co-registered using SPM8, averaged across animals, smoothed and high-pass filtered. GLM was applied to map the BOLD response. **RESULT:** Fig. 1c shows the optogenetically evoked responses in the ipsilateral somatosensory cortices recruited via the stimulation of thalamocortical neurons in VP at 10, 20 and 40Hz respectively. Spatially, 10Hz stimulation evoked responses in both the primary (S1) and secondary (S2) somatosensory cortex compared to evoked responses only in S2 during 20 and 40Hz stimulation. All stimulation frequencies evoked responses locally in VP. Temporally, all the BOLD temporal profiles in S1 and S2 took approximately 6s after onset of stimulation before reaching its first peak. However, the subsequent 14s during stimulation, the BOLD temporal profile decayed for 10Hz stimulation, reached a steady state for 20Hz, and increased in an approximately linear manner for 40Hz. Post-stimulation, the 10Hz and 20Hz BOLD temporal profiles took 10s to decay to baseline while 40Hz took 20s. The same phenomenon was also seen when the most activated voxel from each slice was selected. Fig. 2b and c shows that the optogenetically evoked responses in the ipsilateral barrel cortex (BC) and S1 recruited via the stimulation of Po. The BOLD time profile shows similar characteristics as described in the previous BOLD profile under 20Hz stimulation in VP. No other evoked responses were detected when Po was stimulated with 20 and 40Hz.

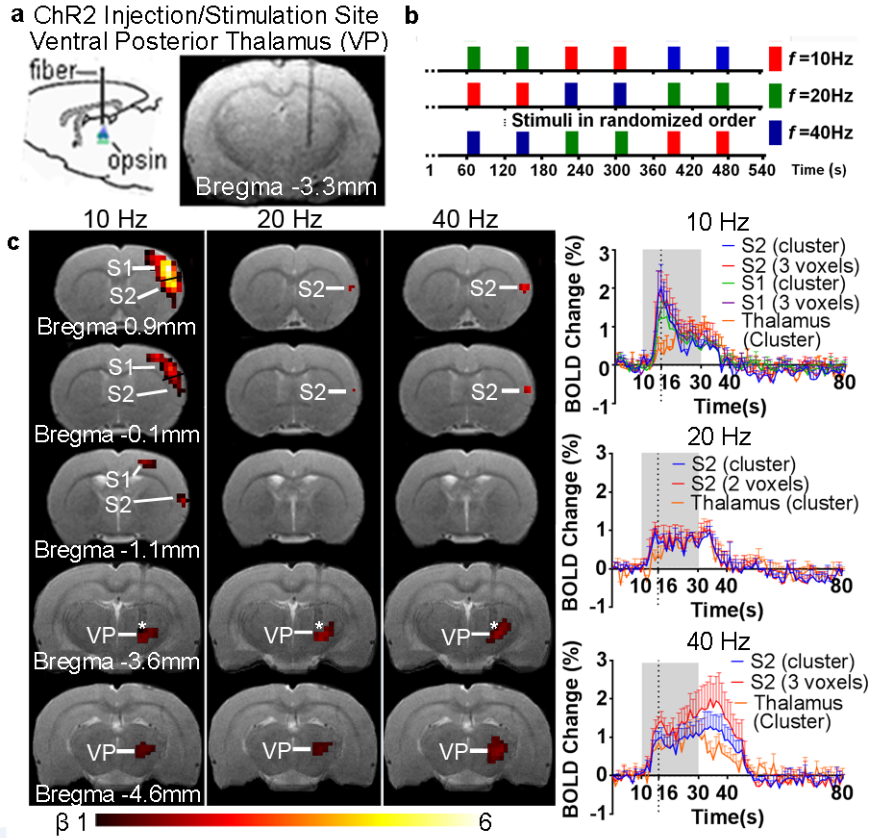

Fig. 1: (**a**) Illustration of stimulation site at ventral posterior nuclei (VP). (**b**) Optical stimulation paradigm with 20s on and 60s off. (**c**) BOLD activation maps at 10, 20 and 40Hz stimulation and the corresponding BOLD temporal profiles (mean + SEM). *, shows the stimulation site at VP. (Primary somatosensory cortex (S1), Secondary somatosensory cortex (S2)).

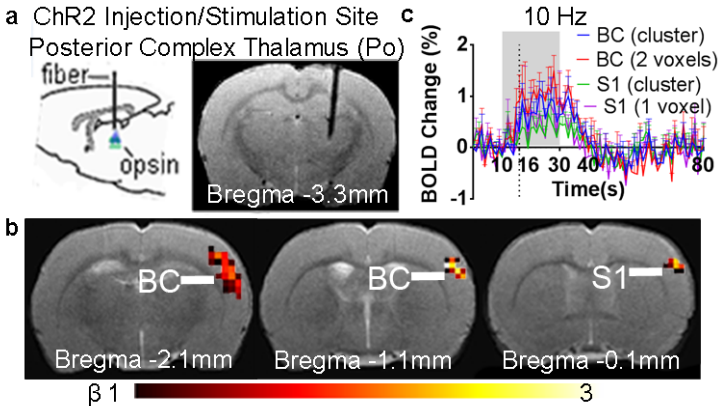

Fig. 2: (**a**) Illustration of stimulation site at posterior complex nuclei (Po). (**b**) BOLD activation maps at 10Hz. (**c**) Corresponding BOLD temporal profile under 10Hz stimulation (mean + SEM). (Barrel cortex (BC), Primary somatosensory cortex, (S1)).

DISCUSSION AND CONCLUSION: Our findings clearly demonstrate the importance of frequency in altering the dynamics and connectivity of the somatosensory thalamocortical circuits. Further experiments are currently underway to examine how different temporal characteristics of stimulation drive thalamocortical circuits differently. The most striking finding so far is that the BOLD time profiles differed widely in the somatosensory cortices. It is plausible that the difference in spatiotemporal BOLD dynamics observed here could be the result of dynamic interactions between the temporally varying stimulation patterns, the intrinsic neural circuitry [3, 4] and ChR2 kinetics [5, 6] when different stimulation frequencies were used. In conclusion, thalamocortical circuits in different subregions respond to different stimulation inputs in a spatially and temporally dynamic manner, indicating the potential ability of stimulation frequency in perturbing specific neural circuits. This opens up future possibilities in devising ofMRI protocols to probe the underlying mechanisms of neural circuits and networks, both locally and globally, in normal and diseased brains in-vivo. Furthermore, our findings also suggest that caution be taken when choosing specific stimulation frequency in future ofMRI studies.

REFERENCES [1] Lee, J.H., et al. Nature, 2010. 465(7299): p. 788-792. [2] Desai, M., et al. J Neurophysiol, 2011. 105(3): p. 1393-405. [3] Steriade, M., et al. The Journal of Neuroscience, 1996. 16(8): p. 2788-2808. [4] Steriade, M. and R.R. Llinás, Physiol rev, 1988. 68(3): p. 649-742. [5] Wang, H., et al. Proc Natl Acad Sci U S A, 2007. 104(19): p. 8143-8. [6] Mattis, J., et al. Nat Meth, 2012. 9(2): p. 159-172.