Intrahippocampal and Hippocampal-Cortical Interactions Driven by Frequency Specific Optogenetic Stimulation

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TARGET AUDIENCE: Researchers studying the intrahippocampal interaction or hippocampal-cortical interaction.

PURPOSE: Hippocampus is one of the most globally connected brain regions [1, 2]. Previous studies have shown that gamma oscillation in the hippocampus is highly synchronized [3], whereas the low frequency oscillation between the hippocampus and the cortex can be coherently related [4]. It has been suggested that the intrahippocampal interactions may be governed by gamma oscillations, while hippocampal-cortical interactions might be directed by low frequency oscillations [3, 4]. However, how oscillation of different frequencies exactly contributes to large-scale intrahippocampal and hippocampal-cortical interactions remains largely unknown. In this study, we applied optogenetic functional MRI (ofMRI) to investigate the frequency-dependent hippocampal network activities in large-scale by driving the CaMKIIa-positive pyramidal neurons in the dorsal hippocampus.

MATERIALS AND METHOD: Animal Preparation: AAV viral vectors (AAV5-CaMKIIa-hChR2(H134R)-mCherry) were injected into the right dorsal hippocampus (-3.5mm anterior/posterior, 2.0mm medial/lateral, -3.5mm and -4.0mm dorsal/ventral from Bregma) of Sprague-Dawley rats (250-300g, N=5). After 4 weeks, a multimode optical fiber with a diameter of 400μm was implanted to the right dorsal hippocampus. ofMRI data were acquired immediately after fiber implantation. **Optical Stimulation:** 470nm blue light (1Hz or 40Hz, 30% duty cycle, 50mW/mm^2) was delivered for stimulation in a block design (Fig. 1). **MRI Protocols:** All MRI data was acquired on a 7T Bruker scanner with a surface coil. ofMRI data was acquired using a single-shot GE-EPI sequence with TR/TE= 1000/20ms, FOV=32×32mm^2, 64×64 matrix and 10 contiguous 1-mm slices. High resolution T2-W images were acquired as anatomical references. **Data Analysis:** Functional images were realigned, coregistered to a template, and smoothed with a Gaussian kernel of 0.5mm FWHM using SPM8. A general linear model analysis was applied to calculate voxel-wise response t-maps. Significantly activated voxels were determined by using a threshold of t>3.11 (equivalent to p<0.001). Images from blocks corresponding to the same frequency were further averaged. The time series of percentage BOLD signal change were extracted and plotted.

RESULTS: For 40Hz stimulation, positive BOLD response was observed in bilateral dorsal hippocampus, while negative BOLD response was observed in the ventral dentate gyrus (vDG) (Fig. 3). For 1Hz stimulation, positive BOLD response was observed in multiple cortical and subcortical regions (Fig. 3). The temporal dynamics of optogenetically evoked responses were examined and are summarized in Fig. 4. In brief, 40Hz stimulation led to a stronger and more prolonged response with faster rise time in the ipsilateral HP than in the contralateral HP. The contralateral HP response also exhibited a longer onset delay (by ~1s) than the ipsilateral HP response. In addition, negative BOLD was observed in the vDG. For 1Hz stimulation, the responses were generally weaker but more distributed across dDG, Rsp, VC and S1. Ipsilateral dDG showed a more prolonged response than Rsp. After reaching the peak, Rsp response decreased faster than dDG. Furthermore, VC/S1 and Rsp exhibited a slower response onset than dDG (by ~2s and ~1s, respectively).

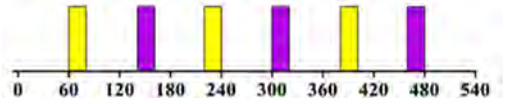

Fig. 1 Interleaved 1Hz (yellow) and 40Hz (blue) optical stimulation paradigm with 20s on and 60s off periods.

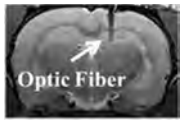

Fig. 2 T2-W images show the fiber implantation targeting the right dorsal hippocampus.

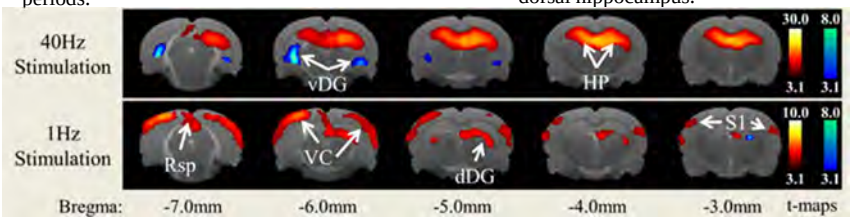

Fig.3 The t-maps corresponding to 40Hz (top), and 1Hz stimulation (bottom). For 40Hz stimulation, positive BOLD response was observed in both the ipsilateral and contralateral dorsal hippocampus (HP), while negative BOLD response was observed in the ventral dentate gyrus (vDG). For 1Hz stimulation, positive BOLD response was observed in multiple cortical and subcortical regions, including bilateral visual cortex (VC), bilateral primary somatosensory cortex (S1), retrosplenial cortex (Rsp), and ipsilateral dorsal dentate gyrus (dDG).

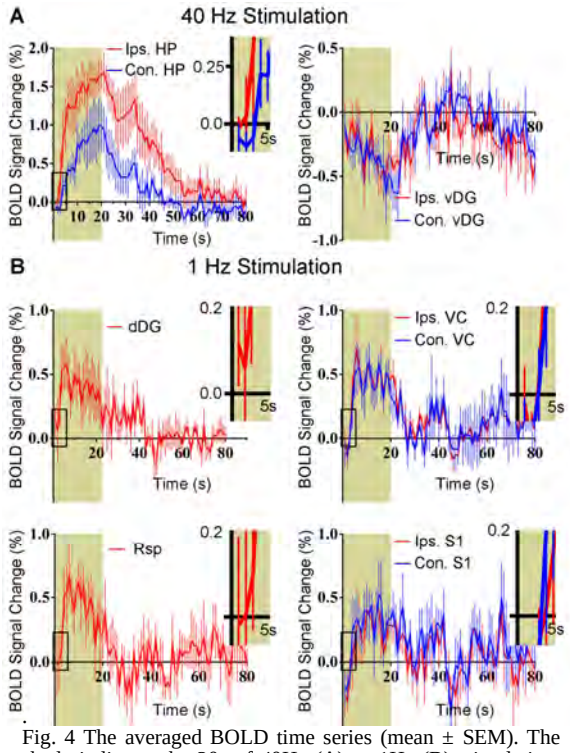

Fig. 4 The averaged BOLD time series (mean ± SEM). The shade indicates the 20s of 40Hz (A) or 1Hz (B) stimulation. The first 5s (box) were zoomed-in and displayed (top-right).

DISCUSSION AND CONCLUSION: Large-scale hippocampal and cortical activations were observed during 40Hz and 1Hz stimulation of CaMKIIa-positive excitatory pyramidal neurons in the right dorsal hippocampus, respectively. These results supported that intrahippocampal interactions and hippocampal-cortical interactions may be governed by gamma oscillations (30-100Hz) and low frequency oscillations (<1Hz), respectively. During 40Hz stimulation, the optogenetically evoked responses should first occur in the ipsilateral HP, and then the neuronal signals propagate via the dorsal hippocampal commissure to evoke responses in the contralateral HP [5]. Thus, it is not surprising that the contralateral HP showed a more delayed and slower response though the mechanism underlying the long extra delay (~1s) remained unclear. The prolonged response observed in the ipsilateral HP might be associated with long-term potentiation [5]. Negative/deactivation BOLD signal has been associated with neuronal inhibition [6]. The negative BOLD observed in the vDG might be related to the mechanism involved in the signal propagation from the ipsilateral to the contralateral HP via dorsal hippocampal commissure, where gamma oscillation-like optogenetic stimulation may overdrive the bilateral DG and may consequently trigger GABAergic inhibition [3,7]. In contrast, 1Hz stimulation caused widespread cortical activations. This likely arose from the hippocampal-cortical connections and slow oscillations induced by slow 1Hz optogenetic stimulation. The origin of the long response onset delay (~2s) observed in VC and S1 remained unclear. It could be related to the specific hippocampal-cortical interactions. The prolonged response observed in the ipsilateral dDG could be associated with long-term potentiation. These 1Hz findings suggested that low frequency stimulation of excitatory neurons in the dorsal hippocampus might generate low frequency oscillation, induce hippocampal-cortical interactions, and modulate cortical activities and oscillation networks. In conclusion, frequency-specific optogenetic stimulation of excitatory pyramidal neurons in the dorsal hippocampus induces large-scale intrahippocampal and hippocampal-cortical interactions. Temporally varying optogenetic stimulation in combination with fMRI presents opportunities to study local and long-range circuits and oscillation dynamics.

REF: 1. Power JD, Neuron, 2013. **2.** Cole MW, Neuroimage, 2010. **3.** Csicsvari J, Neuron, 2003. **4.** Ekstrom AD, Neuroimage, 2014. **5.** Andersen, P., The Hippocampus Book, 2006. **6.** Schäfer K, Neuroimage, 2011. **7.** Coulter DA, Prog Brain Res. 2007.