NeuroImage xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

Optogenetic fMRI reveals distinct, frequency-dependent networks recruited by dorsal and intermediate hippocampus stimulations

Andrew J. Weitz [a,1], Zhongnan Fang [b,c,1], Hyun Joo Lee [c,d,1], Robert S. Fisher [d,1], Wesley C. Smith [e,1], ManKin Choy [d], Jia Liu [e,f], Peter Lin [c,d], Matthew Rosenberg [g], Jin Hyung Lee [a,b,c,d,e,f,h,*]

[a] Department of Bioengineering, Stanford University, CA 94305, USA
[b] Department of Electrical Engineering, Stanford University, Stanford, CA 94305, USA
[c] Department of Electrical Engineering, University of California, Los Angeles, CA 90095, USA
[d] Department of Neurology and Neurological Sciences, Stanford University, CA 94305, USA
[e] Neuroscience Interdepartmental Program, University of California, Los Angeles, CA 90095, USA
[f] Department of Psychiatry and Biobehavioral Sciences, University of California, Los Angeles, CA 90095, USA
[g] Department of Psychology, University of California, Los Angeles, CA 90095, USA
[h] Department of Neurosurgery, Stanford University, CA 94305, USA

ARTICLE INFO

Article history:
Accepted 14 October 2014
Available online xxxx

Keywords:
Functional MRI
Brain mapping
Optogenetics
Hippocampus
Seizure

ABSTRACT

Although the connectivity of hippocampal circuits has been extensively studied, the way in which these connections give rise to large-scale dynamic network activity remains unknown. Here, we used optogenetic fMRI to visualize the brain network dynamics evoked by different frequencies of stimulation of two distinct neuronal populations within dorsal and intermediate hippocampus. Stimulation of excitatory cells in intermediate hippocampus caused widespread cortical and subcortical recruitment at high frequencies, whereas stimulation in dorsal hippocampus led to activity primarily restricted to hippocampus across all frequencies tested. Sustained hippocampal responses evoked during high-frequency stimulation of either location predicted seizure-like afterdischarges in video-EEG experiments, while the widespread activation evoked by high-frequency stimulation of intermediate hippocampus predicted behavioral seizures. A negative BOLD signal observed in dentate gyrus during dorsal, but not intermediate, hippocampus stimulation is proposed to underlie the mechanism for these differences. Collectively, our results provide insight into the dynamic function of hippocampal networks and their role in seizures.

Introduction

Based on a large volume of anatomical, behavioral, and genetic data, it has been argued that the dorsal and intermediate compartments of the hippocampus are two functionally distinct regions (Fanselow and Dong, 2010). This claim is supported by observations of differences along the dorsoventral axis of the hippocampus, including the distribution of cortical afferents and projections (Cenquizca and Swanson, 2007; de No, 1934; Van Groen and Lopes da Silva, 1985), connections with other subcortical structures (van Groen and Wyss, 1990), commissural and intrinsic connections (van Groen and Wyss, 1990), neurochemical makeup (Garcia Ruiz et al., 1993; Tanaka et al., 2012), and cell morphology (Dong et al., 2009). In addition to these physical differences, physiological properties such as spatial field tuning (Jung et al., 1994), vulnerability to ischemia (Ashton et al., 1989), and evoked field potential responses (Gilbert et al., 1985) also differ along the dorsoventral axis. Furthermore, lesion, stimulation, and pharmacological studies have demonstrated distinct behavioral effects when different areas along this axis are targeted (Hock and Bunsey, 1998; Siegel and Flynn, 1968). Nevertheless, despite the wealth of information on these differences, exactly how they contribute to large-scale functional network activity remains unknown. Furthermore, less is known about the intermediate hippocampus, with most studies focusing on the dichotomy between dorsal and ventral regions. We chose to target the intermediate, rather than ventral, hippocampus to study how network properties differ with small changes in distance along the dorsoventral axis.

While anatomical connectivity provides a foundation for studying a region's functional role in neural circuits, understanding how projections translate to activity is best done by directly manipulating the population of interest and observing the downstream effects. Electrical stimulation was traditionally used for this purpose (Canals et al., 2009; Canals et al., 2008), but the recent development of optogenetics has enabled greater precision in the temporal pattern of excitation and the specific subset of neurons targeted (Boyden et al., 2005; Yizhar et al., 2011; Zhao et al., 2011). Electrophysiology measurements

Abbreviations: DH, dorsal hippocampus; IH, intermediate hippocampus.

* Corresponding author at: 1201 Welch Road, #P206, Stanford, CA 94305, USA.
E-mail address: ljinhy@stanford.edu (J.H. Lee).

[1] These authors contributed equally.

http://dx.doi.org/10.1016/j.neuroimage.2014.10.039
1053-8119/

have typically been used to observe downstream effects of stimulation, but are limited by a finite number of recording sites, requiring *a priori* hypotheses about which regions are affected. As one of the few modalities that can report activity across the entire brain with relatively high spatial resolution, functional magnetic resonance imaging (fMRI) offers a viable alternative to studying the network-level behavior of neural circuits in both humans and small animals (Bullmore and Sporns, 2009; Goloshevsky et al., 2008; Huettel et al., 2004; Yu et al., 2010). By measuring the blood oxygenation-level dependent (BOLD) signal over time, fMRI provides an indirect measure of neuronal activity, including both spatial and temporal dynamics (Kim et al., 2004).

Optogenetic functional magnetic resonance imaging (ofMRI) is a novel technology that combines the precision of optogenetic stimulation with the whole-brain readout capability of fMRI (Desai et al., 2011; Lee, 2011, 2012; Lee et al., 2010; Vazquez et al., 2013; Weitz and Lee, 2013). Unlike fMRI experiments with cognitive, sensory, or even direct electrical stimuli, ofMRI can investigate the brain's response to a cell type-specific population being driven in a temporally precise manner, revealing important aspects of a network's connectivity and frequency response *in vivo*. This has enabled investigators, for the first time, to manipulate specific elements of a neural circuit with precision in an intact animal and observe the causal flow of activity within the global brain circuit. By systematically investigating different patterns of input and measuring the brain's response, a region's functional and dynamic connectivity can be characterized. In particular, this technique can be used to investigate how network activity changes with different temporal patterns of input. Several studies employing optogenetics have already demonstrated the distinct frequency responses that neuronal circuits can exhibit (Adamantidis et al., 2007; Carter et al., 2010; Gradinaru et al., 2009). Although several experiments have used ofMRI to examine the functional connectivity of different regions (Abe et al., 2012; Desai et al., 2011; Lee et al., 2010), this aspect of neuronal dynamics has not been explored yet. In addition, several other questions remain unanswered. Can ofMRI detect activity across multiple synapses? Can it dissociate inhibitory and excitatory activities? And can it reveal the spatiotemporal dynamics of large-scale, multi-region networks?

In the present study, we sought to address these issues by using high-resolution, high-field ofMRI to investigate the causal, frequency-dependent network activity driven by CaMKIIa-positive cells in dorsal (DH) and intermediate (IH) hippocampus of rats. CaMKIIa-positive cells in these two regions were selectively stimulated using optogenetic techniques at 6, 10, 20, 40, and 60 Hz during whole-brain fMRI scans. Our results indicate distinct networks recruited by stimulation of either region in a frequency-dependent manner, and point to the potential of ofMRI in uncovering the functional significance of each region in large-scale neuronal networks and behavior.

Materials and methods

Subjects

Adult male Sprague–Dawley rats (250–350 g; Charles River Laboratories, Wilmington, MA) were used as subjects. Animals were individually housed under a 12-hour light–dark cycle and provided with food and water *ad libitum*. Animal husbandry and experimental manipulation were in strict accordance with National Institute of Health, UCLA Institutional Animal Care and Use Committee (IACUC), and Stanford University IACUC guidelines.

Surgical preparations

To achieve targeted control of a single neuronal population in the dorsal or intermediate hippocampus, we injected adeno-associated virus expressing a Channelrhodopsin2-EYFP (enhanced yellow fluorescent protein) fusion protein under control of the Ca^{2+}/calmodulin-dependent protein kinase IIa (CaMKIIa) promoter (expressed primarily in excitatory cells) into either region of the right hemisphere. The pAAV–CaMKIIa–ChR2(H134R)–EYFP plasmid was constructed by cloning CaMKIIa–ChR2(H134R)–EYFP into an AAV backbone using MluI and EcoRI restriction sites (map available online at www.optogenetics.org). The recombinant AAV vector was serotyped with AAV5 coat proteins and packaged by the University of North Carolina viral vector core (titer of 2×10^{12} particles/mL).

During surgery, rats were anesthetized with isoflurane (induction 5%, maintenance 2–3%; Sigma-Aldrich) and secured in a stereotactic frame. Following a midline incision, a small craniotomy and viral injection/cannula implantation were performed at the dorsal hippocampus (−4.3 mm AP, +1.2 mm ML right hemisphere, −3.1 mm from bregma; Figs. 1A and B) or intermediate hippocampus (−5.8 mm AP, +5.2 mm ML right hemisphere, −3.4 mm from bregma; Figs. 1C and D). Two microliters of virus was delivered through a 34-gauge needle (World Precision Instruments Inc.) at 150 nL/min. The syringe needle was left in place for 10 min before being slowly withdrawn. A custom-designed fiber optic cannula was mounted and secured on the skull using metabond (Parkell Inc.), with the fiber optic's end positioned 0.2 mm above the corresponding injection site to ensure adequate illumination of transfected cells. Incisions were sutured, and animals were kept on a heating pad until recovery from anesthesia. Buprenorphine was injected subcutaneously twice daily for 48 hours post-operatively to minimize discomfort. All experiments were conducted at least 3 weeks after virus injection to allow for optimal ChR2 expression.

Upon completion of ofMRI studies, a cohort of the imaged animals underwent additional surgeries for EEG electrode implantation ($n = 3$ DH-injected; $n = 4$ IH-injected). Surgical preparation details were the same as those used for virus injection and cannula placement. Two stainless steel screws (0–80, 1.5 mm diameter, Plastics One Inc.) were attached to ~2 cm of insulated wire (30 gauge, R30Y0100, Wire Wrapping Wire, O.K. Industries) and affixed to the skull over the frontal cerebral cortex (Fig. S1A). A reference electrode was placed approximately 3 mm anterior and 2 mm to the right of bregma. The recording electrode was placed at the edge of the cerebral cortex above the dorsal or intermediate hippocampus approximately 1.5 mm caudal to the optical fiber implant location. Electrodes were mounted on the skull and secured with metabond (Parkell Inc.). Incisions were closed with 5–0 nylon skin sutures. Surgical recovery details were the same as those for virus injection. Animals were also supplied with trimethoprim–sulfamethoxazole antibiotic (48 mg/100 ml) in their water.

ofMRI experiments

fMRI scanning was performed in a 7T Bruker Biospec small animal MRI system at UCLA. Animals were initially anesthetized with 5% isoflurane and intubated before placement onto custom-made MRI-compatible cradles. Intubation was performed according to a protocol from Rivard et al. (2006) by inserting a modified 16- or 18-gauge i.v. catheter into the glottis to serve as an endotracheal tube. A 39 mm outer diameter and 25 mm inner diameter custom-designed transmit/receive single-loop surface coil was centered over the region of interest on the skull to maximize signal-to-noise ratio. An optical fiber of 62.5 μm core diameter was connected to a 473 nm laser source and coupled with the implanted cannula.

During fMRI scanning, animals were placed into the iso-center of the magnet while artificially ventilated (45–55 strokes/min) under light anesthesia using a ventilator (Harvard Apparatus, Model 683 Small Animal Ventilator) and calibrated vaporizer with a mixture of O_2 (35%), N_2O (63.5%), and isoflurane (1.2–1.5%). Expiratory CO_2 was kept at 3–4%, and body temperature was maintained at 36–38 °C using heated air-flow. T2-weighted high-resolution anatomical images were acquired prior to fMRI scanning to check for brain damage and validate the optical fiber's location. Gradient recalled echo (GRE) BOLD methods were used to acquire fMRI images during photostimulation. The fMRI image

Please cite this article as: Weitz, A.J., et al., Optogenetic fMRI reveals distinct, frequency-dependent networks recruited by dorsal and intermediate hippocampus stimulations, NeuroImage (2014), http://dx.doi.org/10.1016/j.neuroimage.2014.10.039