Optogenetically induced BOLD of excitatory neurons in the mouse hippocampus at 9.4T: identification of a hippocampal network

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Introduction

Recently Lee et al. [1] demonstrated in rats that BOLD signals in a particular region can be caused by activation of local excitatory neurons through optogenetic stimulation of the motor cortex. Since it is known that hippocampal-prefrontal network plays an important role in many major psychiatric disorders like depression and schizophrenia, we were first interested in the hippocampal network in mice. To selectively stimulate the hippocampal population of excitatory neurons, we induced expression of channelrhodopsin-2 (ChrR2) in Ca2+/calmodulin-dependent protein kinase II (CAMKII)-expressing neurons. For this purpose a Cre-recombinase-dependent adeno-associated viral vector was unilaterally injected into the hippocampus of transgenic mice expressing Cre recombinase under the control of the CaMKII promoter (CAMKII:Cre). The injected area was later stimulated by a pulsed laser in a fMRI block-design experiment at 9.4T. Here we present the preliminary results of one of the first animals measured in this study.

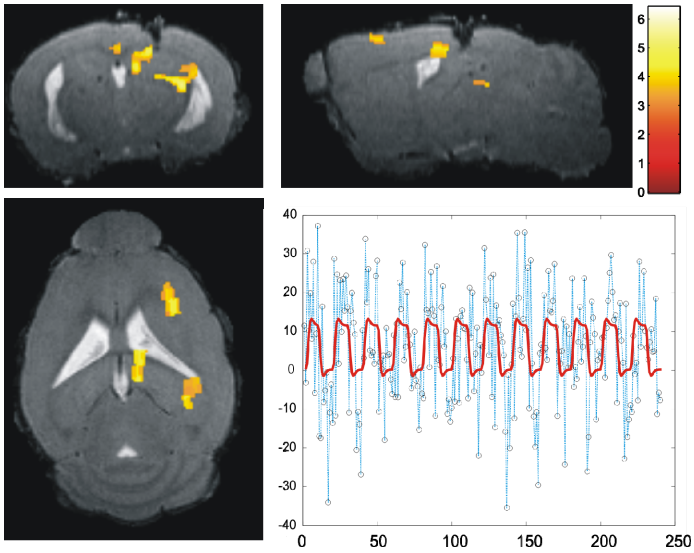

Fig. 1 Activation maps of the laser block design at p<0.001 uncor. (min. 10 voxel/cluster) overlaid on a coregistered anatomical 3D-dataset with the predicted response and time course of the most significant voxel in the latero-caudal CA3 region of the left hippocampus (radiological orient.).

Methods

We used a double-floxed inverted open reading frame strategy with two nested pairs of incompatible lox sites [2] In Cre-expressing cells, ChR2-YFP (yellow fluorescent protein) is first reversibly flipped into the sense orientation via either pair of sites; this enables a second irreversible excision that prevents further inversion. Double-floxed reversed ChR2–YFP was produced and virus (300nl) was injected stereotactically into the left dorsal hippocampus of 12-16-week-old CAMKII:Cre mice, a transgenic mouse line [3] that was back-crossed to C57BL6 (Charles River). A guidance canula was implanted above the site of viral infection, and sealed with a dummy cap. During the fMRI experiments, animals were anaesthetized with 1.6% isofluorane. A tailored fiber optic (0.15 mm outer diameter, OZ Optics) was introduced into the guidance canula. Imaging was conducted in a 9.4T animal scanner (Bruker, Germany) equipped with a linear transmit volume resonator and an anatomically shaped surface receive coil for the mouse brain. After obtaining a high-res. T_2-weighted anatomical 3D-dataset (RARE, 78x78x156µm), fMRI data was acquired using a 12-repetition block design with a segmented EPI Sequence (12 slices, 128x128 matrix, 156x94x500µm res., TE 12ms, TA 3s, 240 volumes). Photostimulation was delivered by a 473nm laser (CrystaLaser) at a power of 1-3 mW/mm2 pulsed at 20Hz (5ms pulse width). Laser stimulation was periodically applied for 30 sec through the optical fiber at 1 min intervals. Image preprocessing consisted of coregistration to the anatomical dataset, motion correction and smoothing with a 4 mm isotropic Gaussian kernel. The smoothed EPI images were analyzed in a GLM with SPM5 using a HRF-convolved box car function as a main regressor including time, dispersion and movement parameters as covariates. To validate spatial distribution of ChR2:YFP expression after completion of the experiment, 50-um thick coronal brain slices were prepared.

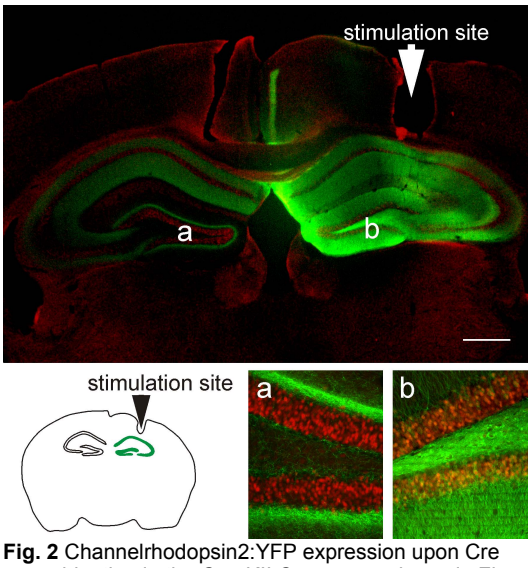

Fig. 2 Channelrhodopsin2:YFP expression upon Cre recombination in the CamKII:Cre mouse shown in Fig. 1: ChR2:YFP expression (green) and Cre expression (red). In the site of infection (b) Cre positive cells in the dentate gyrus also express ChR2:YFP, whereas on the contralateral site (a) ChR2:YFP is predominantly found in fibers (scale bar 0.5 mm).

Results

ChR2:YFP postive cells were predominantly observed below the site of photostimulation in the dentate gyrus and in adjacent CA3 region, ChR2:YFP positive fibers were observed throughout the ipsilateral and contralateral hippocampal formation (Fig. 2). The statistical maps in Fig.1 displays activation nearby the laser-targeted region: positive BOLD response was observed in the medio-rostral parts of CA1 and a large part of CA3 of the targeted hippocampus. Interestingly, we also found a corresponding significant activation in areas outside the stimulated region: in the primary somatosensory cortex (S1), the primary motor cortex (M1) and in a less extent, in the basal retrosplenial granular zone.

Discussion

The observed activation pattern, which in addition to the hippocampus included also the primary motor cortex and the somatosensory cortex, could be explained by the hippocampal excitatory inputs to these cortical regions [4]. This may constitute a functional network, which could provide spatial navigation and motor control through the exchange of information between these structures. To our knowledge this is the first study demonstrating that hippocampal-cortical networks can be specifically identified by means of optogenetic fMRI. Because of the importance of the hippocampal-prefrontal network in many psyhiatric disorders, future studies will assess other specific neuronal circuits and their possible frequency-dependent interactions with and within this network.

References :

[1] Lee JH et al. Nature 2010 465(7299):788-92. **[2]** Sohal VS et al. Nature 2009 459(7247):698-702. **[3]** Mantamadiotis T et al. Nat. Genet. 2002 31(1):47-54. **[4]** van Strien NM et al. Hipp. 2009 10:272-82.