The Influence of Laser Power and Pulse Characteristics on the Optogenetically Induced BOLD Signal of Excitatory Neurons in the Mouse Hippocampus at 9.4T

Philipp Lebhardt[1], Wolfgang Kelsch[2,3], Claudia Falfan-Melgoza[1], Gabriele Ende[1], Alexander Sartorius[2], and Wolfgang Weber-Fahr[1]

[1]*NeuroImaging, Central Institute of Mental Health, Mannheim, Germany,* [2]*Clinic of Psychiatry and Psychotherapy, Central Institute of Mental Health, Mannheim, Germany,* [3]*Cinical Neurobiology, University of Heidelberg, Hiedelberg, Germany*

Introduction

In this study we were interested to investigate the effects of laser heating and different stimulation frequencies in optogenetic BOLD imaging of the mouse hippocampus [1]. To selectively excite the hippocampal population of excitatory neurons, we induced expression of channelrhodopsin-2 (ChrR2) in Ca2+/calmodulin-dependent protein kinase II (CAMKII)-expressing neurons [2] (Fig 1a). Additionally to investigate the laser heating effect on the BOLD signal we measured sham animals without virus injection.

Methods

We investigated the left dorsal hippocampus of 12-16-week-old CAMKII:Cre+ mice with a double-floxed reversed CHR2-mcherry virus and in sham animals without virus injection. In all scanned mice a guidance canula was implanted for optical fiber (0.1mm outer diameter) insertion in the left hippocampus. During imaging freely breathing mice were anesthetized with dormitor. Imaging was conducted in a 9.4T animal scanner equipped with a linear transmit volume resonator and an anatomically shaped surface receive mouse brain coil. The fMRI data was acquired using a 12 repetition laser stimulation block design with an EPI sequence (17 slices, 96x96 matrix, TE 16ms, TR 1s). In the first paradigm the photostimulation was periodically applied for 30s through the fiber at 1min intervals. In the sham animals, the laser power was altered stepwise between four scans from 4.8mW (average pm=1.4mW) to 19.2mW (pm=5.7mW), all pulsed at f=20Hz (15ms pulse duration) and a wavelength λ=473nm. In the animals with virus injection we modulated the character of the photostimulation from scan to scan at P=4.8mW with pulse durations of 15ms and 5ms.

To investigate the differences between prolonged block activation and event related burst signals and to measure the event related BOLD response in mice, the second paradigm consisted of short 1s activation "bursts" in 10s intervals at a frequency of 40Hz (5ms pulse duration) with a laser power of 4.8mW and 14.3mW respectively. Three burst intervals are followed by a 30s rest period.

The image preprocessing consisted of correction for physiological noise [3], coregistration to anatomical dataset, motion correction and smoothing with a 0.4mm isotropic Gaussian kernel. The smoothed EPI images were analyzed in a GLM with SPM8 using a box car function convolved with the hemodynamic response function (hrf) as a main regressor including movement parameters as covariates. To acces the mouse-hrf in the event related design we used a Finite Impulse Response (FIR) basis function (8s length, order 8).

Results

In the sham mice we found a laser heating effect of increasing deactivation with increasing laser power in the areas close to the fiber optic and activation in more distant areas (Fig. 1b-i). According to these results the laser power was set to 4.8mW for the stimulation in mice with virus injection. Stimulation at 20Hz yielded a high significant activation ipsilateral for pulse duration of 15ms and 5ms with higher BOLD signals at shorter pulse duration (Fig. 2a,b). Interestingly the burst excitation (Fig. 2c,d) indicated a highly significant activation in the contralateral hippocampus for both laser power level and in addition an ipsilateral activation for P=14.3mW. The FIR analysis of the BOLD signal response to a burst activation shows that the hemodynamic response function in mice reaches maximum signal already after 3s, considerably shorter than in human measurements. The burst paradigm also showed some secondary activation in the retropsplenial and infralimbic cortex at p<0.001 uncor. (Fig. 2f).

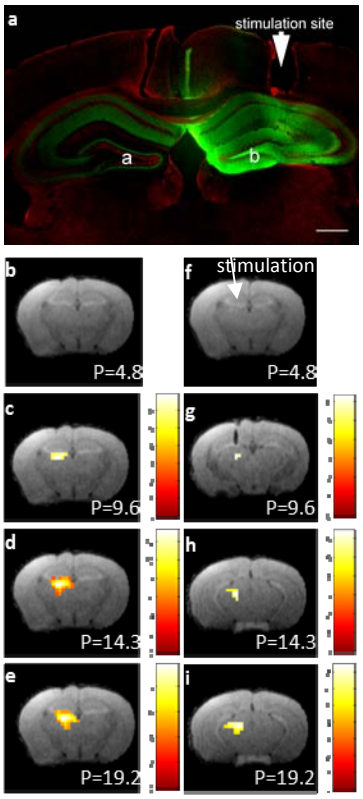

Fig.1: a) Channelrhodopsin2:MCH expression upon Cre recombination in the CamKII:Cre mouse. ChR2:MCH expression (green) and Cre expression (red) (rad. view) b)-e) deactivation and f)-i) activation in sham mouse for different laser power p<0.05FWEcorr.

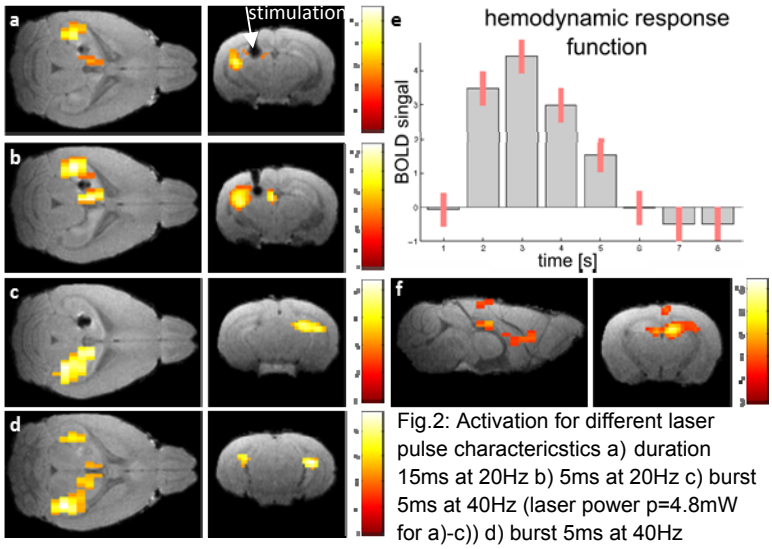

Fig.2: Activation for different laser pulse characterictics a) duration 15ms at 20Hz b) 5ms at 20Hz c) burst 5ms at 40Hz (laser power p=4.8mW for a)-c)) d) burst 5ms at 40Hz p=14.3mW (p<0.05FWEcor.) e) hemodynamic BOLD response (FIR) f) Acivation of retrosplenial and infralimbic cotex after burst 5ms/40Hz activation (p<0.001uncor.)

Discussion

Our measurements show that at Laser power greater than 4.8mW (pm=1.4mW) strong heating effects are visible in the MR-BOLD signal for prolonged stimulation.

Also the pulse durations, frequencies and paradigm types not only change the strength of the BOLD activation, but also considerably the pattern of the activated networks. The generally higher activation with shorter laser pulse duration and especially the burst activation could be explained by an adaption process of the neuronal networks through prolonged optogenetic activation.

References **[1]** Lee JH et al. Nature 2010 Jun 10;465(7299):788-92. **[2]** Weber-Fahr W et al. Proc ISMRM 2011 19:4182. **[3]** van Buuren et al. Human Brain Mapping 2009 30:3031-3042