Investigation of Optogenetically Induced Oxytocin Release within Central Amygdala on BOLD Signals in Rat Brain at 9.4T

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Introduction:

In this abstract we are presenting preliminary results on a study investigating the oxytocinergic network. To selectively excite oxytocin neurons and their axons we expressed Channelrhodopsin-2 (fused with m-Cherry) driven by 2.6kb oxytocin promoter by recombinant adeno-associated virus (rAAV) [1]. We examined the effect of laser stimulation in the central amygdala in female virgin rat brains with optogenetic BOLD imaging. Only animals, in which blue light activation of oxytocin release from axons in the central amygdala prevented the conditioned freezing response (see the protocol in [1]) were scanned in a 9.4T animal scanner to study the BOLD response to the laser stimulation.

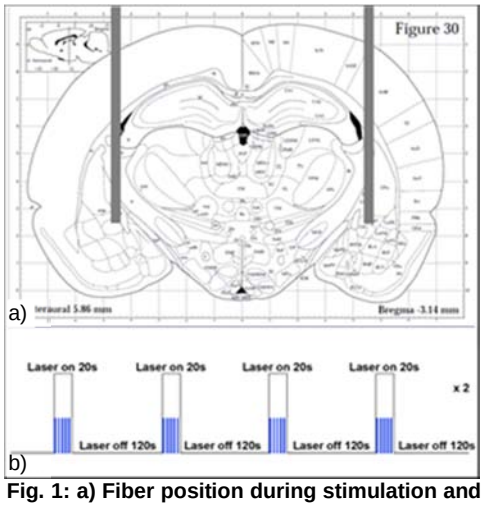

Fig. 1: a) Fiber position during stimulation and fMRI scanning; b) stimulation paradigm 20s (t_D=5ms, f=30Hz) stimulation followed by 120s pause

Method:

Adult female virgin rats were stereotaxically injected with the rAAV into hypothalamic nuclei (paraventricular, supraoptic and accessory nuclei) to express Channelrhodopsin-2-mCherry driven by 2.6kb oxytocin promoter in oxytocin neurons as described [1]. After delivering rAAV into the brain, a guided cannula was implanted into the central amygdala for stimulation with blue light (Fig. 1Fig. 1a). Three to four weeks later the rats were subjected to fear conditioning protocol [1, 2]. Afterwards rats that showed a substantial reduction of fear response after blue light-evoked axonal oxytocin release within the central amygdala were further subjected for fMRI scanning. Imaging was conducted in a 9.4T animal scanner equipped with a linear transmit volume resonator and an anatomically shaped surface receive rat brain coil (Bruker, Ettlingen, Germany). Before scanning the rats were anesthetized with medetomidine (bolus: 0.5ml (0.07mg/kg), continuous infusion: 0.14mg/kg/h). The fMRI data was acquired using an eight repetition block design with an EPI sequence (T_R=1.7s, T_E=17.5ms, 29 slices with spatial resolution: 0.365x0.365x0.5mm^3). During the scanning the laser stimulation was applied bilaterally in the central amygdala periodically for 20s followed by a 120s pause (Fig. 1b). The laser pulse duration was t_D=5ms with a pulse frequency f=30Hz and a laser power P=10mW (this corresponds to an average laser power during stimulation period of P_{av}=1.5mW) at a wavelength of λ=473nm. Functional data processing was done with SPM8 including correction for physiological noise with Aztec [3].

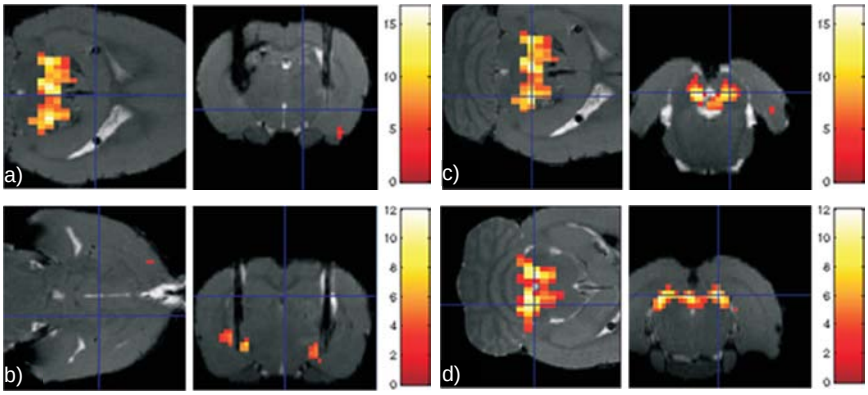

Fig. 2: BOLD activation surrounding the stimulation areal a) rat 1 b) rat 2; Position of highest BOLD signal change in supra colliculus and pag for paradigm 1 (20s bilateral laser stimulation followed by 120s pause) for c) rat 1 and d) rat 2 (p<0.001uncor., k=5vox)

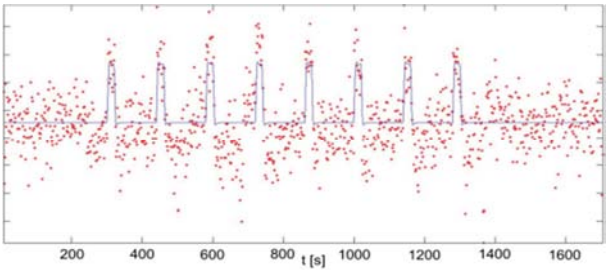

Fig. 3: Mean timecourse of BOLD activated cluster in colliculus area of rat 2 for laser stimulation of 20s followed by 120s pause during scanning.

Results:

In the central amygdala rat 1 showed no BOLD signal change (p=0.001uncor., k=5Vox) (Fig. 2a). The highest BOLD signal change was detected in the superior colliculus area and periaqueductal grey (PAG) in this animal (Fig. 2c). In the second rat a bilateral BOLD signal increase in the stimulation area was recognized (Fig. 2b), superior colliculus area and PAG showed a distinct BOLD activation (Fig. 2d). The mean time-course of the activated cluster in the superior colliculus area showed a clear dependence of BOLD signal change and laser stimulation (Fig. 3).

Discussion:

In spite of the small number of blue-light activated oxytocinergic terminals detected in the central amygdala [1], a strong BOLD activation in dorsal midbrain, a center for the control of the freezing response [4], was detected in both rats. This result confirms indirect and direct projections from central amygdala to PAG and superior colliculus [5].

References: [1] Knobloch S. et al., Neuron 73, 553–566, 2012; [2] Meloni E. G. and Davis M., Behav. Neurosci. 113, 1152-1160, 1999; [3] van Buuren M. et al., Hum. Brain Mapp. 30, 3031–3042 (2009); [4] Zhao Z. and Davis M., J. Neurosci. 24, 10326-10334, 2004; [5] Rizvi T.A. et al., J. Comp. Neurol. 303, 121-131, 1991.