GABAergic effect on resting-state functional connectivity

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Purpose: Resting-state synchrony of fMRI signals between brain regions indicates functional connectivity (FC) between them [1]. However, the neurobiological mechanisms giving rise to the synchronous oscillations are not clear [2]. Associations between regional neurotransmitter concentrations and BOLD signal are suggested [3,4]. Therefore challenging neurotransmitter receptor systems pharmacologically may shed some insight into the neural substrates of resting-state FC. Since a drug may also change the neurovascular coupling, it complicates the interpretation of the BOLD signal [5]. We investigate the effects of challenging the GABAergic system with the $GABA_A$ receptor antagonist, bicuculline, on task activation and resting-state FC and correlated with EEG.

Methods: Male Wistar rats (290-360g) were used in this study. All animals were orally intubated and mechanically ventilated under 3% isoflurane and then maintained at 1.3% for fMRI and EEG experiments with pancuronium bromide (1.5mg/kg i.v.). Bicuculline (dose = 0.06 mg/kg/h) or equivalent volume of vehicle was administered i.v. The study was approved by the local Institutional Animal Care and Use Committee. ***fMRI***: MRI were performed using a 9.4T scanner (Agilent, USA). In ten rats, activation by electrical forepaw stimulation and resting state were investigated before and under bicuculline/vehicle infusion. Electrical pulses of 9 Hz, 0.3 ms duration, and 2, 3, and 4 mA current were given to both forepaws following a block design with 60s resting and 20s stimulation alternating three times. Cross-correlation was used to detect the activation. Resting state fMRI constituted a 50 min continuous scan, 10 min baseline and 40 min under bicuculline infusion. All data was acquired using single-shot spin-echo EPI (TR 2 sec, TE 38 ms, 1 mm slice thickness, 64x64 matrix size, and FOV 2.56x2.56 cm). The processing of the resting state data included high-pass filtering at 0.01Hz, and spatial smoothing with a FWHM of one pixel. The average signals from the ventricles and skin were regressed out to reduce contributions from physiological noises. A 2x2 pixel ROI in the left SI forepaw region was chosen to detect the functional connectivity. ROI's were also taken in the ipsilateral visual cortex (VC) and contralateral hippocampus (Hipp). In both studies, a correlation coefficient higher than 0.25 was considered significant and clusters smaller than 4 pixels were rejected. ***Electrophysiology***: For EEG measurements (n=14), the rat's head was secured in a stereotaxic frame, the skull exposed and two holes were drilled at left and right SI forepaw areas (3.5mm lateral, 1mm anterior of the bregma) and electrodes were inserted at 1mm below dura. Another electrode was inserted at 10mm posterior to the right hole as the reference. Resting EEG was recorded with a high pass filter at 0.01Hz, sampled at 1k Hz for 600s (MP150, Biopac, USA). Somatosensory evoked potential (SEP) was recorded under 30s resting followed by 10s stimulation. Integral of the SEP was calculated. For resting EEG, we compute the coherence between both SI, as well as the power distribution in different frequency bands.

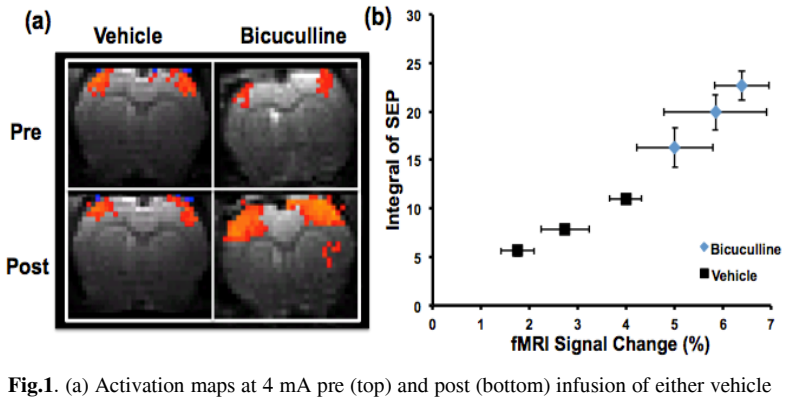

Fig.1. (a) Activation maps at 4 mA pre (top) and post (bottom) infusion of either vehicle (left) or 0.06mg/kg/h bicuculline (right). (b) Correlation between the BOLD fMRI signal change and SEP of vehicle (black) and bicuculline (blue) under forepaw stimulation.

Results: As expected, the $GABA_A$ antagonist bicuculline increased neural activation as evident from the SEP and BOLD. Both measures were highly correlated indicating similar neurovascular coupling (Fig.1). Significant and gradual increase in FC was detected over time (Fig. 2a,b). The increase in connectivity was seen in VC, but not in regions with low $GABA_A$ receptor density, eg, Hipp (Fig. 2b). Strong correlations were found between BOLD connectivity and EEG coherence in both beta ($p<0.00063$) and gamma ($p<0.00059$) bands under bicuculline but not vehicle (Fig. 2c).

Discussion: Antagonizing the $GABA_A$ receptor with a dosage that is not inducing seizure results in a strong increase in task activation and resting-state FC. Especially, inter-regional connectivity throughout the cerebral cortex was increased. Connectivity with regions of high $GABA_A$ receptor density like VC was seen, but not striatum and thalamus which may partly be due to the effect of anesthesia. The increase in EEG coherence is consistent with the known role of GABA on beta and gamma oscillations [6]. The results suggest GABAergic system could modulate long-range FC and the neural basis of resting-state BOLD synchrony.

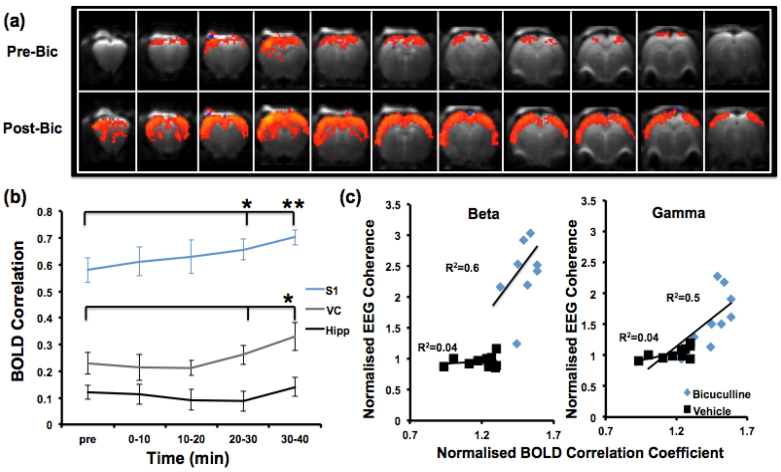

Fig. 2. (a) Resting state connectivity maps of SI region before (top) and under (bottom) infusion of 0.06 mg/kg/h bicuculline. (b) Connectivity with SI, VC, and Hipp changes over every 10 min intervals during bicuculline infusion. (c) Correlation between BOLD correlation in S1 and EEG coherence in the beta and gamma bands under vehicle

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