Resting-State Functional Connectivity in Rat Brain

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Introduction

Synchronized low-frequency fluctuations in resting-state fMRI (1) have been used to investigate functional connectivity between brain areas. Recent studies demonstrate that this technique can be utilized in the study of Alzheimer's disease (2), antidepressant effects (3), and "default mode" of brain networks (4). So far, most resting-state fMRI studies have been performed on humans, with fewer studies on animals reported. This is probably due to difficulties related to animal studies, including anesthesia effects on the resting-state signals and potential impact of faster cardiac cycling in small animals. However, animal models may offer important advantages for understanding the underlying mechanisms of resting-state signals. For instance, combined electrophysiological recordings with resting-state fMRI measurement could help interpret the temporally correlated signals between functionally related brain areas. We report here the measurement of resting-state functional connectivity on rats at 9.4T using contrast-agent (ferumoxtran-10) based functional signal, with the assumption that the influence of cardiac pulsation through blood is minimal due to very short T_2 of venous blood under this condition.

Methods

Resting-State Data Acquisitions. Experiments were performed on a Bruker 9.4T scanner. Four SD rats (250~300 g) were anesthetized with α-chloralose (an initial dose of 50 mg/kg followed by 50 mm/kg each hour) and mechanically ventilated. Rectal temperature, endtidal CO_2, O_2, and arterial blood pressure were continuously monitored and kept within normal ranges. Superparamagnetic contrast agent Combidex® (ferumoxtran-10, Advanced Magnetics, Cambridge, MA) were administrated (I.V.) at an iron dose of 15 mg/kg. Resting-state data were acquired using a gradient-echo EPI sequence, with FOV = 3.5 cm, slice thickness = 1.5 mm, matrix size = 64×64, TE = 15 ms, and TR = 1 sec. A total of 270 volumes were collected in 270 sec. For comparison, a block-design forepaw stimulation (consisting of three cycles of 1 min "off" and 1 min "on") was delivered using a Grass-88 stimulator, with pulse duration of 3 ms, frequency of 3 Hz and current intensity of 3 mA. Functional MRI data with stimulation were collected in 320 sec.

Data Processing and Analysis. For fMRI data acquired with forepaw stimulation, activated pixels were identified using cross correlation (cc >0.4, $p<10^{-3}$). The resting-state fMRI data were processed with a low-pass digital filter (0.1 Hz) to suppress high-frequency components. Cross correlation was then used to evaluate temporal correlation between brain areas. Seed voxels were selected from the stimulus-induced activation maps. The threshold for cross-correlation (>0.4) was used to obtain functional connectivity maps.

Results

Fig.1 illustrates representative results of stimulus-related activation maps and resting-state functional connectivity maps obtained from a rat. As expected, increased activity is shown in the contralateral forepaw somatosensory cortex (Fig.1a). Using a voxel selected from the activation map as a seed point, functional connections between the somatosensory cortices on both hemispheres is clearly shown in the resting-state functional connectivity maps (Fig.1b).

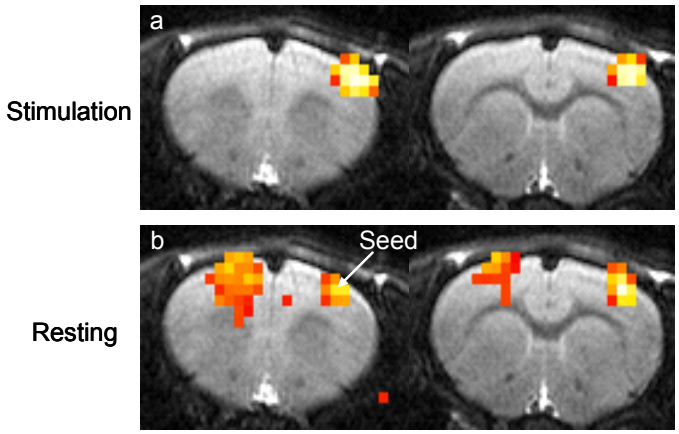

Fig.1 (a) Activation maps obtained from the fMRI data acquired with single-sided forepaw stimulation. (b) Functional connectivity maps obtained from the resting-state data.

Discussions

We have demonstrated that resting-state fMRI is feasible in rats anesthetized with α-chloralose, although the dosage effects on functional connectivity are not known. A recent study showed a reduction of functional connectivity with increasing concentrations of sevoflurane anesthesia (5). It is worthwhile to further investigate the influence of anesthesia level and type on resting-state fMRI.

Since the cardiac rate of rats is about 350 per min, which is much higher than the image acquisition rate (TR=1s), artificial signals associated with cardiac cycling could affect the resting-state functional signals. In this study, we used a superparamagnetic contrast agent (ferumoxtran-10) to reduce signals contributed from the blood, and thus to minimize potential confounds from cardiac pulsations. The T_2 of venous blood at 9.4T is only 9ms (6) and is reduced to less than 5ms after injection of Combidex (>5mg/kg), therefore, signal contributed from blood in the EPI images (acquired at TE of 15ms) is negligible. Furthermore, the influence of cardiac pulsation, if any, would be global in the connectivity maps, which is clearly not seen in the results.

Previous animal studies using laser Doppler flowmetry demonstrated that cerebral blood flow (CBF) waves are associated with patterns of electro-cortical activity (7). Our study is primarily based on cerebral blood volume (CBV) contrast. Since CBF and CBV changes are usually closely coupled, the observed low-frequency CBV fluctuations in this study may arise from the same sources of synchronous neuronal activities as observed in previous CBF studies.

References

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