Resting-state functional connectivity of primary somatosensory cortices in urethane anesthetized rats

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INTRODUCTION

Spontaneous low frequency fMRI fluctuations measured during resting-state have been shown to provide important information on spontaneous neuronal activity and the functional organization of the brain [1]. Although many resting-state fMRI studies have been performed on human subjects, only a handful of studies have been conducted in rat models. The major concern in rodents and in animal studies in general is the use of anesthesia. Previously medetomidine hydrochloride (Domitor) anesthesia has been reported to be feasible for resting-state fMRI experiments in rats [2,3,4]. Urethane anesthesia, on the other hand, has been shown to be suitable for fMRI and local field potential (LFP) experiments [5], but to our knowledge the feasibility of urethane for resting-state fMRI studies has not been evaluated. In this study, we measured fMRI from rats under urethane anesthesia during electrical forepaw stimulation and resting-state in order to evaluate the feasibility of urethane for resting-state fMRI experiments in rodents. The electrical forepaw stimulation was used to functionally locate the region of interest, primary somatosensory cortex (SI), which was used as the "seed" region for calculating resting-state correlation maps. SI was chosen as the region of interest based on the previously reported bilateral correlation of these areas in resting-state under Domitor anesthesia and because the functional location of SI can be robustly detected with fMRI using electrical forepaw stimulation.

METHODS

Five male Wistar rats (314-360 g) were first anesthetized with isoflurane after which urethane (1.25 g/kg, *i.p.*) was administered and isoflurane discontinued. A pair of small needles (30 G) was introduced into the skin of the right forepaw for stimulating the somatosensory cortex. Functional MRI acquisition was typically started 1-2 hours after changing anesthesia, when the influence of initial isoflurane anesthesia can be considered to be negligible.

The MRI measurements were performed using a 4.7 T horizontal scanner (Magnex Scientific) interfaced with a Varian Unity^{Inova} console. An actively decoupled volume radiofrequency coil and quadrature surface coil pair (RAPID Biomedical GmbH, Rimpar, Germany) was used for signal transmission and reception. The functional imaging slice was positioned coronally based on sagittal pilot scans to the somatosensory cortex at bregma. Functional MRI data were measured using a single shot spin echo EPI sequence (TR 2 s, TE 60 ms, slice thickness 1.5 mm, 64x64, FOV 2.5x2.5 cm). First, an fMRI data set during electric stimulation of the right forepaw (electrical pulses of 0.3 ms duration, 1.2 mA, repeated at 10 Hz frequency) was acquired for locating the left primary somatosensory cortex (30 images of baseline, 15 images of activation, repeated three times and adding 30 images of baseline at the end) after which a resting-state data set of 300 scans was collected without any external stimulation. Anatomical images were acquired in the end of the experiment using multislice spin-echo sequence (TR 2.5 s, TE 70 ms, 256x256, FOV 5x5 cm).

The electrical forepaw stimulation data set was analyzed using SPM5 (Wellcome Department of Imaging Neuroscience, University College London, UK) and the resulting activation map was used to determine the location of the 2x2 voxel seed region in the primary somatosensory cortex (SI). The preprocessing of the resting-state data included global intensity normalization, spatial smoothing with a 2x2 voxel Gaussian kernel, removing linear trend and low-pass filtering all time series with a cutoff frequency of 0.08 Hz. The average time course of the seed region in SI was then extracted from the preprocessed resting-state data and cross-correlated (Pearson's correlation coefficient) with every voxel time course in the brain. A correlation coefficient value higher than 0.3 ($P<10^{-6}$) was considered significant and clusters smaller than 4 pixels were rejected. Finally, the anatomical images were overlaid with the corresponding thresholded correlation coefficient maps (Fig. 1B).

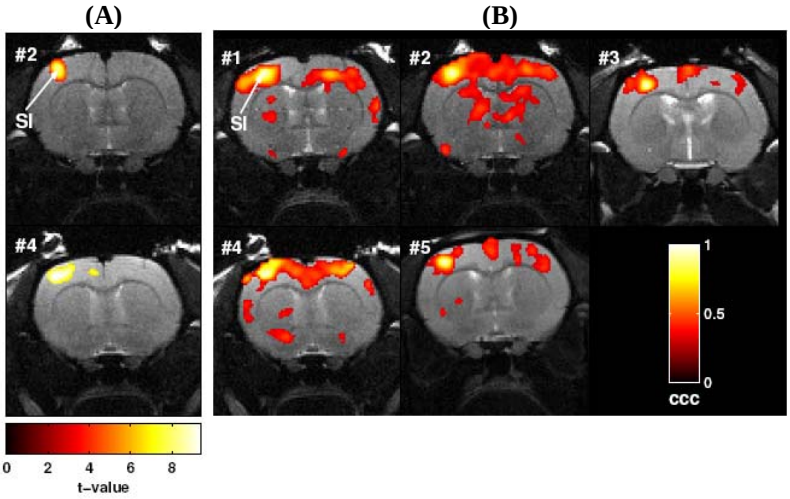

Fig. 1. fMRI activation maps in response to electrical forepaw stimulation for two typical rats (A) and resting-state functional connectivity maps of five rats (B) under urethane anesthesia. Left primary somatosensory cortex (SI) was functionally identified using electrical forepaw stimulation and used as the seed region of interest for cross-correlation analysis of the resting-state fMRI data. Cross-correlation coefficient (ccc) values higher than 0.3 ($P<10^{-6}$) were considered significant.

RESULTS

Figure 1 (A) shows the activation of left primary somatosensory cortex (SI) in response to electrical stimulation of the right forepaw for two rats and (B) the resting-state functional connectivity maps for five rats under urethane anesthesia. Significant correlation of low frequency BOLD fluctuations is seen between left (seed region) and right SI areas. This bilateral coupling of SI is seen in all five rats.

DISCUSSION

In this study, the feasibility of urethane anesthesia for studying resting-state fMRI in rats was evaluated. Previous studies in rats have shown that there exists a strong correlation of low frequency fMRI fluctuations between somatosensory cortices under Domitor anesthesia [2,3,4] and our findings indicate that this bilateral coupling between hemispheres is also preserved under urethane anesthesia. Urethane produces a long-lasting level of surgical anesthesia and preserves cardiorespiratory function along with intense skeletal muscle relaxation, which is very useful for fMRI experiments. Medetomidine hydrochloride, on the other hand, does affect cardiorespiratory function by lowering the respiration rate, which in some cases can cause problems. Although urethane is not feasible for longitudinal recovery experiments it does not influence electrophysiology and can be used for simultaneous fMRI and local field potential (LFP) measurements.

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