Effects of Anesthesia on Resting-State Functional Connectivity in Rat Brain

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Introduction

Functional connectivity in the brain can be assessed using synchronized low-frequency fluctuations in resting-state fMRI (1). Most resting-state fMRI studies have been conducted on humans, and several have examined anesthesia effects on connectivity between brain regions (2,3). The paucity of animal studies is likely due to such methodological difficulties as the potential impact of faster cardiac rates. However, animal models may offer important advantages for understanding the underlying mechanisms of resting-state signals. We have recently reported feasibility of measuring resting-state functional connectivity in rats at 9.4T using contrast agent-based functional signal. Contrast agent could shorten blood T_2 substantially and thus minimize the effects of cardiac pulsation through blood (4). In the present study, we further examine the anesthesia effects of α-chloralose on resting-state brain connectivity and externally-induced functional activation in rats.

Methods

Data Acquisition. Experiments were conducted on six α-chloralose anesthetized SD rats using a Bruker 9.4T scanner. A loading dose of 80 mg/kg (I.V.) was administered by bolus injection. Continuous infusion (30 mg/kg) was initiated 30 min after the loading dose. The second (70 mg/kg) and third (100 mg/kg) doses of α-chloralose were administered manually (I.V.), with a time interval of approximately 50 min. Image acquisition was started 15 min after the beginning of the 30 mg/kg continuous infusion, and the 70 and 100 mg/kg bolus injection, respectively. Animals were artificially ventilated. Rectal temperature, end tidal CO_2, O_2, and arterial blood pressure were continuously monitored and kept within normal ranges. Superparamagnetic contrast agent (Ferumoxtran-10, Advanced Magnetics, Inc.) was administrated (I.V.) at an iron dose of 15 mg/kg. Three sets of resting-state and forepaw stimulation data were collected after each α-chloralose infusion. Resting-state data were acquired using a gradient-echo EPI sequence, with FOV of 3.5 cm, 7 slices with thickness of 1.5 mm, matrix size of 64×64, TE of 15 ms, and TR of 1426 msec. A total of 270 volumes were collected in approximately 385 sec. For comparison, a block-design forepaw stimulation experiment was performed (Grass-88 stimulator), with pulse duration of 3 ms, frequency of 3 Hz and current intensity of 3 mA. Forepaw stimulation data were collected in 210 sec using the same parameters as in resting-state fMRI (except TR of 1s).

Data Processing and Analysis. Both forepaw stimulation and resting-state fMRI data were slice-timing corrected, volume registered, linear detrended and spatially smoothed (FWHM of 0.8752 mm) using AFNI. For forepaw stimulation data, relative signal change between activation and baseline was calculated based on the stimulation paradigm for each rat. For resting data, a low-pass filter with cutoff frequency of 0.1 Hz was applied to suppress physiological noise. The map of cross-correlation coefficients (CC) was obtained by cross-correlating each voxel's time course with the average time course of seed voxels, which were selected based on forepaw stimulation map. The CC map was then converted to a z-score map. To enable group analysis, all rats' anatomical images were affine-transformed into the first rat's anatomical space by defining 7 anatomical markers and the same transformation was later applied to each rat's relative signal change maps and z-score maps at each of the three doses. ANOVA analysis was then performed on these registered signal change maps and z-score maps.

Results

Forepaw stimulation generated significant brain activation on the contralateral somatosensory cortex at all three doses of α-chloralose, although functional signal changes were reduced as a function of dose. The average signal change in the activated region was 2.09±0.69, 0.92±0.49 and 0.74±0.32 at 30 mg/kg, 70mg/kg, and 100 mg/kg α-chloralose, respectivley. The signal change at 30 mg/kg was significantly higher than at 70 and 100 mg/kg ($p < 0.005$).

Fig.1 shows group functional connectivity maps from 2 slices of rat brain superimposed on T2 anatomical images when seed voxels were selected in the right somatosensory cortex based on the forepaw stimulation data. Functional connection is clearly seen in the ipsilateral (right side) somatosensory cortex (relative to the seed voxels) at all three α-chloralose doses, and on the contralateral side at lower doses. The average CCs of the significantly connected areas in the ipsilateral somatosensory cortex were 0.21±0.06, 0.16±0.03, and 0.18±0.04 at α-chloralose doses of 30, 70 and 100 mg/kg, respectively. There was no significant difference in CC between these groups. The average CCs of the significantly connected areas in the contralateral somatosensory cortex were 0.18±0.08, 0.11±0.05, and 0.09±0.06 at doses of 30, 70 and 100 mg/kg, respectively, and the CC at dose of 30 mg/kg was significantly higher than those at 70 and 100 mg/kg ($p<0.05$).

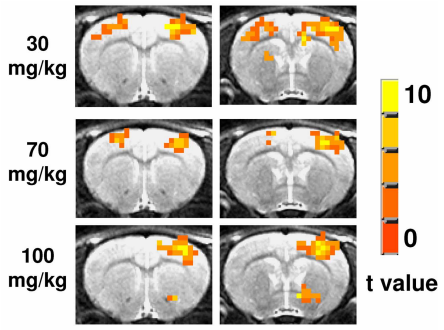

Fig.1 Group functional connectivity maps at different doses of α-chloralose (n=6).

Discussions

There are known callosal connections between the dysgranular zones of the left and right S1, which originate from pyramidal neurons in layers 3 and 5 and terminate in homonomous layers of the contralateral hemisphere. Our data suggest that functional connectivity between S1FL of both hemispheres persist during α-chloralose anesthesia, and can be modulated by anesthetic dose. These findings are consistent with a recent study showing a reduction of functional connectivity with increasing concentrations of sevoflurane anesthesia in humans (3). Cortical EEG in cats is altered at different anesthesia stages and deeper anesthesia resulted in profound neuronal inhibition and functional disorganization (5). Such neuronal inhibition may contribute to dissociations between brain regions, leading to a reduction in functional connectivity as observed at higher doses of α-chloralose in our data. Further work is needed to elucidate the neuronal basis of such functional connectivity.

Fast cardiac rates in small animals (~400 per min in rats) could potentially affect the resting-state functional signals. In this study, we used a superparamagnetic contrast agent (Ferumoxtran-10) to reduce signal contributed from blood, and thus minimize potential confounds from cardiac pulsations. The T_2 of venous blood at 9.4T is reduced to less than 5ms after injection of Combidex (>5mg/kg), and therefore signal contributed from blood in the EPI images (acquired at TE of 15ms) was negligible as confirmed by the specificity of brain regions in the connectivity maps.

References: 1. Biswal et al, Magn Reson Med 1994; 34:537-541. 2. Kiviniemi et al, Magn Reson Med 2000; 44:373-378. 3. Peltier et al, NeuroReport 2005; 16: 285-288. 4. Lu et al, ISMRM 2005; p.532. 5. Winters, Ann Rev Pharmacol Tocicol 1976; 16:413-426.

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