Inter-hemispheric Resting State Functional Connectivity in Anesthesia Induced Unconsciousness

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Introduction: The neural mechanism underlying anesthesia-induced unconsciousness (AIU) remains elusive (1). In this study, we aimed to investigate the system level circuit mechanism of AIU by analyzing the inter-hemispheric resting-state functional connectivity (RSFC) at the awake state and five different anesthesia depths. Pairwise T Test was utilized to reveal the connectivity changes at various anesthetized states. In addition, the inter-hemispheric RSFC was correlated to behavioral data across all AIU states.

Method: Animals were firstly acclimated to the scanner environment to minimize the motion and stress level during imaging at the awake state using the paradigm developed in our lab. Resting-state fMRI data of 9 male Long-Evans rats at the awake as well as anesthetized states induced by 0.5%, 1.0%, 1.5%, 2.0% and 3.0% isoflurane were acquired on a 7T scanner interfaced with an Agilent console. The behavioral test using righting reflex indicated that awake, transitional and unconscious states were induced in animals at these isoflurane doses. rsfMRI data were collected using single-shot EPI (TR=1s, TE=13.78ms, FOV=3.2*3.2cm, flip angle is 60°, matrix size is 64×64 and 20 slices of 1mm thickness, 400 volumes each scan). rsfMRI data were co-registered to a standard rat atlas, motion corrected using SPM, and then spatially and temporally smoothed. In addition, signals from white matter and ventricles, as well as six motion parameters were regressed out. To evaluate inter-hemispheric connectivity, the whole brain was parcellated to 68 bilateral regions of interest (ROIs) and each ROI was further divided into 2 unilateral (left and right) ROIs. Regionally averaged ROI signals from individual pairs of unilateral ROIs were extracted and used to calculate inter-hemispheric RSFC. Before imaging experiment, the righting reflex test was carried out in individual animals. The latency to right was used to test the conscious level at each isoflurane dose. The max time for the right reflex test was set to 60s.

Results: As isoflurane dose increased from 0.0% (i.e. awake) to 3.0%, the inter-hemispheric RSFC averaged from all ROIs in general decreased (Fig.1). Three distinct states of consciousness could be identified based on this RSFC measure. The first state included the awake and anesthetized conditions at 0.5% and 1% of isoflurane. The second state included the anesthetized condition at 1.5% and 2.0% isoflurane. The third state included the anesthetized condition at 3.0% isoflurane. The mean inter-hemispheric RSFC was similar within each state while significantly different between three states as revealed by pairwise t-testes (Table 1). Figure 2 shows the inter-hemispheric RSFC strength for individual ROIs. Figure 3 shows the latency to right during the righting reflex test. This behavioral data suggested that animals were fully conscious at 0% and 0.5% isoflurane, fully unconscious at 2.0% and 3.0% isoflurane, and in a transitional state at the 1.0% and 1.5% isoflurane. There was a strong inverse Pearson correlation between the averaged latency to right and mean inter-hemispheric RSFC across all six conditions (r = -0.868, p = 0.025).

Figure.2: The number at the leftside of each row is the slice number, and the row order is from posterior to anterior brain. Figures in each column are derived from data at different isoflurane levels which are noted on the top. Color shows the inter-hemisphere ROI correlation intensity. VIS, visual area. ENT, entorhinal area. CA3, field CA3. SSp, primary somatosensory area. MOp, primary somatomotor area. VISC, visceral area.

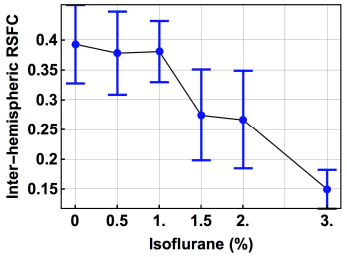

Figure.1: The inter-hemispheric RSFC averaged from all rats at different isoflurane levels. Bars are std.

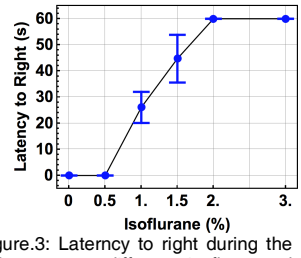

Figure.3: Laterncy to right during the right reflex test at different isoflurane doses. Bars are std.

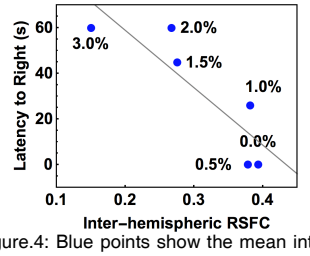

Figure.4: Blue points show the mean inter-hemisphere RSFC and mean latency to right at the same isoflurane dose.

Conclusion and Discussion: Table 1 and Figure 1 clearly indicated that the mean inter-hemispheric RSFC at six conditions could be separated to three stages. This connectivity measure well coincided with the behavioral assessment as shown by a high RSFC-behavior correlation (Fig.4). However, we observed that the RSFC change lagged relative to behavioral manifestation particularly at the isoflurane dose 1% and 2%. We conjectured that this mismatch might suggest that the inter-hemispheric RSFC was a secondary effect for AIU. It has to be noted that not all ROIs followed exactly the same trend. Examples of these ROIs included raphe complex, CA3, entorhinal area, agranular insular area and visceral area (Fig.2). In addition, some ROIs had relatively higher inter-hemispheric connectivity at 1.5% and 2% isoflurane levels, such as visual, ventral temporal association, primary somatosensory and primary somatomotor areas (Fig.2). Interestingly, ROIs in dorsal sensory-motor system had relatively higher inter-hemispheric connectivity than ventral areas especially at 1.5% and 2.0% isoflurane dosages.

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References: 1. Liang et al., J. Neuroscience, 32(30): 10183-10191.

P-value	0.0%	0.5%	1.0%	1.5%	2.0%	3.0%
0.0%	1.0	0.64	0.66	0.0027	0.0022	2.6×10^{-8}
0.5%	0.64	1.0	0.92	0.0084	0.0066	1.2×10^{-7}
1.0%	0.66	0.92	1.0	0.0032	0.0026	4.0×10^{-9}
1.5%	0.0027	0.0084	0.0032	1.0	0.83	0.00037
2.0%	0.0022	0.0066	0.0026	0.83	1.0	0.0011
3.0%	2.6×10^{-8}	1.2×10^{-7}	4.0×10^{-9}	0.00037	0.0011	1.0

Table.1: Pairwise T Test for the averaged inter-hemispheric RSFC at different isoflurane doses.