The Relationship between Level of Consciousness and Variability of Brain Connectivity

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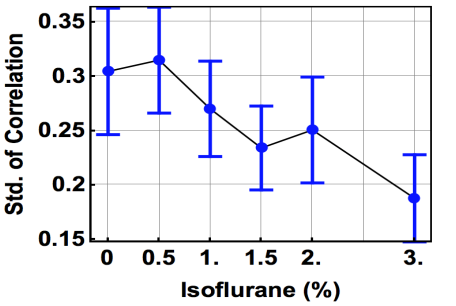
Introduction: Anesthesia is a state of consciousness that can be achieved by altering the level of arousal and/or awareness using anesthetic agents (1,3,4). The systems-level mechanisms of anesthesia are largely unknown due to the complex nature of anesthetic action on neural and vascular substrates (2) as well as the highly variable nature of experimental design (3,4). One variable that deviates among studies is the depth of the anesthesia (3,4). In the present study, we demonstrate that resting-state functional magnetic resonance imaging (rsfMRI) coupled with behavioral measures of anesthetic depth in rats can be used to investigate mechanisms of anesthesia-induced unconsciousness.

Method: Animals were first acclimated to the scanner environment for 7 days to minimize stress and motion during imaging at the awake state. rsfMRI data of 12 male Long-Evans rats were obtained on a 7T scanner interfaced with an Agilent console during six doses of isoflurane anesthetic: 0% (i.e. awake state), 0.5%, 1.0%, 1.5%, 2.0%, and 3.0% (1-shot gradient echo EPI, TR=1s, TE=13.78ms, flip angle=60º, matrix size=64 x 64, FOV=3.2 x 3.2cm, 20 1mm thick slices, in plane resolution=500um x 500um). Between each dose, 5 minutes were provided to assure the dosage reached steady state. Functional images were preprocessed with conventional procedures including registration to a segmented rat brain atlas, motion correction, spatial smoothing, regression of motion parameters and white matter/ventricle signals, as well as band-pass filtering (0.0085-0.1Hz). The distribution of correlations between 68 ROIs across six levels of isoflurane were calculated and correlated with behavioral data obtained with loss of righting reflex (LORR) procedure. In the LORR procedure, each rat was placed in an induction chamber and exposed to one of six doses of isoflurane for 10 minutes. The rat was then turned over to a supine position and observed while measuring the time it took to correct its position. If the rat did not correct its position within the first minute, it was deemed completely unconscious. For each rat, three measurements at each dose were obtained and averaged. LORR data was averaged across all rats per dose (shown in Fig. 4).



P-value	0.0%	0.5%	1.0%	1.5%	2.0%	3.0%
0.0%	1.0	0.64	0.12	0.0020	0.022	9.3×10^{-6}
0.5%	0.64	1.0	0.027	0.00018	0.0038	5.3×10^{-7}
1.0%	0.12	0.027	1.0	0.044	0.32	0.000086
1.5%	0.0020	0.00018	0.044	1.0	0.37	0.0087
2.0%	0.022	0.0038	0.32	0.37	1.0	0.0023
3.0%	9.3×10^{-6}	5.3×10^{-7}	0.000086	0.0087	0.0023	1.0

Fig.2: Standard deviation of correlation for six doses of isoflurane (above) & associated p-value table (below).

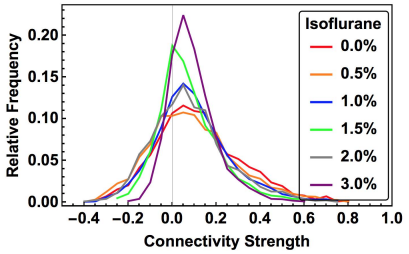


Fig.1: Relative frequency plotted against connectivity strength for six doses of isoflurane.

Results: The distributions of connectivity strength between all 68 ROIs at different isoflurane levels is shown in Fig. 1. In the awake states (0 and 0.5% isoflurane), the distribution shows decreased relative frequency and increased variance compared to our deepest anesthetic doses (1.5 and 3.0% isoflurane), which exhibits significantly lower variability. When plotted, we see that the standard deviation of the correlation decreases as isoflurane dose increases (Fig. 2). This trend in variance is present in some ROIs and not others (Fig. 3). Most importantly, the behavioral data from the LORR procedure (Fig. 4) demonstrates the behavioral effects of isoflurane and is highly correlated with the standard deviation of the correlation (Fig. 5, r=-0.900, p=0.014).

Conclusion and discussion: The main finding that standard deviation decreases as anesthetic depth increases suggests that brain activity becomes more "normalized" in the unconscious state. Previous literature has suggested that there are fewer unique patterns of brain activity during anesthesia-induced unconsciousness (4). We speculate that the decreased variability seen during higher doses of isoflurane may reflect a decrease in the repertoire of brain states that are available to rats during unconsciousness. This research provides novel insight into the relationship between rsfMRI data and behavioral measures. Further analyses of ROI-specific sensitivities to isoflurane may provide more insight into the mechanisms of anesthesia-induced unconsciousness.

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References: 1. Brown et al., Annu Rev Neurosc 34:601-628 2. Masamoto & Kanno J Cerebral Blood Flow & Metabolism 32, 1233-1247. 3. Nallasamy & Tsao, The Neuroscientist 17(1):94-106. 4. Alkire, Hudetz, Tononi, Science, 322:876-880.

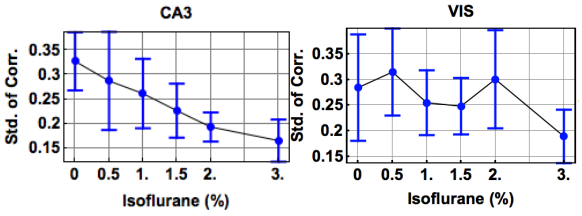

Fig. 3. Standard deviation of the correlation in two example ROIs A) CA3 of the hippocampus and B) visual areas.

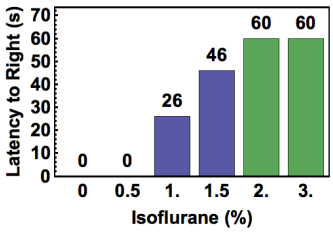

Fig. 4. Averaged latency for rats to right themselves at six doses of isoflurane

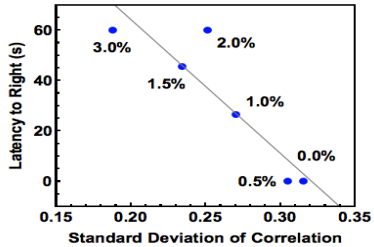

Fig. 5. Correlation between the standard deviation and the latency for rats to right themselves at six doses of isoflurane.