Can time under anesthesia affect resting state connectivity?

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Introduction: Resting-state imaging analyzes the low frequency fluctuations of the BOLD signal (0.01-0.1 Hz) when brain is at rest. It is accepted that these fluctuations reflect neuronal activity (1,2) and also a certain degree of structural (3) and functional connectivity (4). It is known that resting states are reproducible in other mammalian species (5) and that they are not affected by light sleep (6) or even sedation (7). Results for rodents are obtained regularly under anesthesia and apparently the anesthesia has no effect on resting-states (8,9) even if other authors establish that there should exist anesthetic confounds (10, 11). No study tackles the effect of experimental time under anesthesia on resting-state activity. In this study, we compared resting states in two scenarios, one before and after an fMRI experiment and the other when no fMRI experiment was performed between the resting state measurements. Our results show that there is decay in correlation strength depending on the total time under anesthesia; however, this decay does not seem to imply a change in the nature of the correlations.

Methods:
Animal preparation: A group of 14 Sprague-Dawley rats were anaesthetized during experiments with Isoflurane at concentrations between 1% and 1.3%. They were divided in two cohorts of 7 animals. *Physiological monitoring:* Respiration rate (64±9rpm), temperature (37±1˚C), O_2 saturation in blood (98.3±2%), blood pCO_2 level of (38 mmHg ± 10%) and Heart rate (200 ± 30 bpm) were stable during all the experimental time. *Trimming:* All whiskers except the C1-C4 on both sides of the snout were trimmed. *Paradigm:* Group 1 underwent a resting-state study followed by an fMRI experiment and finished with a second resting state study identical to the first one. Group 2 underwent both resting-state studies but lied in the scanner unaltered and un-stimulated during the fMRI experimental time. fMRI stimulation: Whisker stimulation of all remaining right-whiskers was performed at 7Hz for 8s followed by a 24s period of rest. 100 repetitions of the stimulation protocol were performed (1600 whole brain volumes, 22 slices per volume, 53min per fMRI experiment). *MRI:* Scanning was performed on a 4.7T horizontal magnet with a transmit 4 channel array receive surface coil (both Bruker Corp.). Resting state studies used an GE EPI sequence (TR=2s, FOV:25×25 mm, matrix:64×64, 1 mm slice thickness) that acquired 300 brain volumes over a period of 10 minutes. fMRI-BOLD images were acquired with the same GE-EPI sequence within 53 min. An anatomical image set, using a RARE sequence, was obtained after the second resting-state acquisition, parameters were: TR=3000ms, TE=51ms FOV:25×25mm, matrix:256×256, 1 mm slice thickness. *Correlation analysis:* Analysis was performed with a custom made application in IDL (MagNan). After correcting resting-state data for motion, images were smoothed with a Gaussian kernel of 3 voxels followed by a low pass filter of 0.1Hz. 125 regions were segmented in the brain and 6 voxels around the center of mass of each region were selected. If the time profiles of these voxel groups showed high correlation with the stimuli protocol (FDR correction, $q<0.05$) they were kept, otherwise they were eliminated from the analysis. A smaller study was performed on regions known to be related to whisking (4) including bilaterally: Primary Somatosensory (SI), secondary Somatosensory (SII), primary Motor cortex (MI), secondary Motor cortex (MII), Caudate Putamen (CP), Globus Pallidus (GP) and Ventral Posteromedial Thalamic nucleus (VPM). Warm colors indicate high correlation while black and cold colors indicate low correlation in correlation color graphs.

Results: In figure 1 the correlation matrixes (whisker system, first two rows) visually show that the level of correlation is larger for the resting state measurements acquired before (left) the fMRI experiments than after (right). No statistical differences between the two before experiments of Group1 and Group2 were found when comparing the summated correlation values; the same applies to the after experiments ($p>0.700$, t-test, in both cases). The large matrices shown in the bottom row of figure1 indicate a decrease of correlation when all areas of the brain are considered. **Table 1** quantifies these results showing the added correlation values for the large matrix (125 regions) and the whisker matrix (14 whisker regions) for resting-states before and after fMRI. When comparing the before and after experiments for Group1 there was a 10% difference that was statistically significant ($p<0.05$, t-tests) in both cases. Similar results were found for Group 2 with a decrease of 10% ($p<0.03$, t-tests) in both cases.

Discussion & Conclusions: Our results show a significant decrease of correlation between all brain regions with time under anesthesia. This drop is not produced by sensory stimulation during the fMRI experiment as there is no fMRI in Group2 but a similar drop. Physiology was carefully monitored and was stable during experiments therefore not being a possible source for the decrease. In general the pattern of the correlations throughout the brain seems to be unaltered. Consequently the influence of anesthesia seems to be the only sources of this decrease in correlation strength. This work points out the fact that when working with anesthetized animals to study resting states, the time when images are acquired is relevant and has to be considered for comparative experiments.

References: **(1)** Raichle M.E. et al. PNAS 2001 Jan 16;98(2):676-82. **(2)** Lu H., et al. PNAS 2007 104(46): p.18265-9 **(3)** Greicius M.D. et al. PNAS 2003 Jan 7; 100(1):253-8. **(4)** Pawela C.P. et al. Neuroimage 49 (2010) 2467-78. **(5)** Shmuel, A., Leopold, D.A. Hum Brain Mapp 2008, 29, 751-761. **(6)** FukungaM., et al. Magn. Reson. Imaging 2006, 24, 979-992. **(7)** Kiviniemi V., et al. Neuroimage 2003, 19, 253-260. **(8)** Zhao F., Neuroimage 2008 January 1;39(1):248-260. **(9)** Pawela CP. Et al. 2009 Jul 15:46(4):1137-47 **(10)** Ruskin et al. J. Neurophysiology 1999 May;81(5):2046-55. **(11)** PlosOne. 2011;6 (10):Epub 2011 Oct 18.

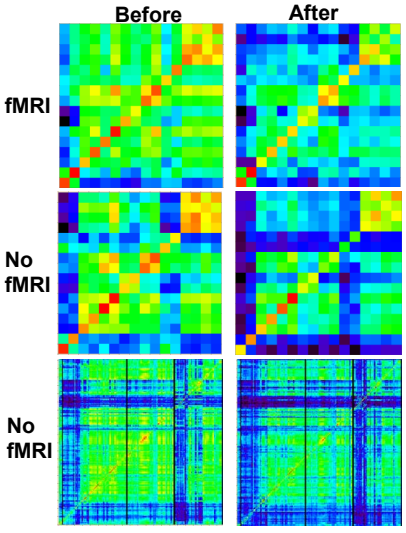

Figure 1. Correlation matrices between brain structures of rat. Warm colors indicate high correlations while cold colors indicate low correlation. First and second row correspond to the matrix of 14 whisker related areas. The third row corresponds to matrixes containing all 125 structures segmented from rat's brain. Row one corresponds to Group1 data (fMRI experiment between resting-states). Row two and three correspond to Group2 (no fMRI experiment but silence between the resting state measurements).

Before	Large Matrix	Small Matrix
Group 1	92,34	7,35
Group 2	93,21	7,47

After	Large Matrix	Small Matrix
Group 1	82,98	6,65
Group 2	82,33	6,67

Table 1. Added correlation values for large (125 regions) and small (14 regions) matrixes. This table presents total addition of correlation values for the two types of matrixes that are presented in this study as well as for both experimental groups.