Mapping of cortico-cortical and cortico-subcortical alterations in functional connectivity induced by light sedation with propofol.

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Introduction Resting state functional magnetic resonance imaging (rs-fMRI) involves measuring the blood oxygen level dependent (BOLD) signal while subjects are at rest, not performing goal-directed tasks. Functional connectivity (FC) analyses of rs-fMRI data aim to elucidate, low frequency (usually <0.1 Hz) BOLD, signal fluctuations between sets of brain regions that form as coherent networks at rest [1]. Sedation is a pharmacologically induced, reversible state, characterized by dose-related impairment of cognitive functions, including attention and memory, but during which consciousness and awareness are maintained [2]. A number of recent studies have been focused on the alterations of FC induced by different levels of sedation [3-5], paying specific attention to the behaviour of regions belonging to the default mode network (DMN) [1]. Because molecular, animal, and human studies have converged upon disruption of thalamocortical communication as a key component of anesthetic-induced unconsciousness [6], we tested for changes in cortico-cortical and cortico-subcortical FC, using regions of interest that encompass major subdivisions of the cortex (Frontal, Temporal, Parietal, Occipital) [7]. We show in this study that the substantial lack of alterations of DMN during propofol induced light sedation is associated with a consistent connectivity alteration between cortical and subcortical regions.

Methods Fifteen healthy right-handed subjects (all males, mean age 28±8 years) participated in this study after giving informed consent. All the participants underwent two fMRI scans, one before and one during propofol administration. *DRUG ADMINISTRATION:* Propofol was administered using a computer controlled infusion pump at dose that produced mild sedation (measured as level 3-4 on a modified "Observer's Assessment of Alertness and Sedation" scale). The average targeted propofol infusion was 1.2±0.2 ug/ml. For the duration of the scan pulse oximetry, electrocardiography (recorded continuously) and non-invasive blood pressure (recorded every five minutes) were monitored. *MRI:* Functional data were collected by using a gradient-echo echo-planar imaging at 3T (GE HDx) using a BOLD weighted imaging sequence (TR=3 s, TE=35 ms, matrix=64x64, FOV/slice=20.5 cm/3.2 mm, flip=90°, 50 slices, 160 vols). A T1 weighted whole-brain structural scan was also acquired (1x1x1 mm voxels). End-tidal carbon dioxide ($PetCo_2$) and end-tidal oxygen ($PetO_2$) traces were recorded throughout the experiment using a nasal canula attached to a gas analyser (AEI Technologies, PA). Cardiac and respiratory processes were monitored using the scanner's built-in photoplethysmograph and a pneumatic belt, respectively. *PREPROCESSING:* Several sources of physiological variance were removed. For each subject, physiological noise correction consisted of removal of time-locked cardiac and respiratory artifacts (two harmonics of each and an interaction term were included) and of low-frequency respiratory and heart rate effects [8]. In addition regressors formed from end-tidal CO_2 and O_2 traces were removed [9]. Several steps of ordinary preprocessing followed: correction for head motion and slice-timing, intensity normalization and non brain voxels removal (performed using FSL: FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl). At this step, using custom software written in Matlab, we regressed out the six parameters obtained by realignment and band-pass filtered data in the frequency range (0.01-0.1 Hz). Finally data were non-linearly registered to a standard space (Montreal Neurological Institute MNI152 standard map) using FNIRT (an FSL routine) and spatially smoothed (5x5x5 fwhm). *DATA ANALYSIS:* Four regions of interest (ROI) were identified [7] (Frontal, Parietal, Temporal Occipital), by thresholding to 0.7 the related probability MNI structural maps (Figure 1). For each subject the mean timecourse (in the standard space) of the preprocessed rs-fMRI data was calculated within each ROI. Each timeseries was used as a regressor in a linear regression model, thus returning four ROI-specific connectivity maps. To examine the influence of Propofol sedation on the group level statistics, a higher level analysis (FLAME) was run. Paired comparisons were performed to identify areas where functional connectivity varied significantly with the neurophysiological state (awake vs sedated). To determine statistical significance of the observed activation while controlling for multiple comparisons, Z statistic images were thresholded using clusters determined by Z >2.3 and a (corrected) cluster significance threshold of p<0.05.

FIGURE 1

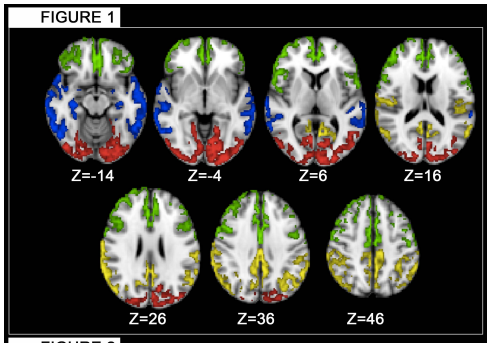
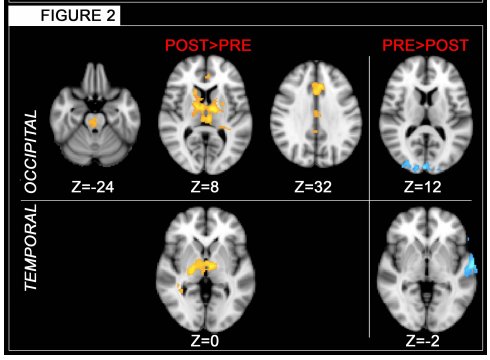


FIGURE 2



Results Frontal and Parietal functional connectivity analyses did not show any significant differences between awake and sedation conditions, at the threshold Z>2.3 (p < 0.05 cluster corr). Conversely Temporal and Occipital regions showed alterations of connectivity in the sedated condition. In particular their connectivity was reciprocally reduced by the effect of propofol (pre>post). While the Temporal cortex shows a reduction of connectivity with the visual cortex, after propofol injection, at the same time Occipital cortex shows a decrease of connectivity with the left temporal lobe (Figure 2, bottom). For the contrast post>pre the Temporal cortex showed an increase of connectivity with the brainstem, the cingulate cortex (especially the anterior portion) and the portion of thalamus connected to the pre-frontal cortex. Interestingly, also the Occipital cortex showed an increased connectivity with the pre-frontal portion of the thalamus (Figure2, top). For the sake of completeness we investigated the connectivity alterations, induced by the mild sedation, relaxing the conservative statistical threshold used above, and we found (data not shown) a one to one decrease of connectivity of the Parietal Cortex both with Occipital and Temporal cortices (p<0.005 uncorr).

Discussion and Conclusions Consistently with results present in literature [5] we reported an interesting systems level mechanism of functional disconnection between cortical regions induced by sedation. In fact, if on one hand we confirm the almost unchanged structure of the DMN connectivity under sedation [3,4] (we didn't find any significant functional disconnection between ROIs containing nodes belonging to the DMN), on the other hand we found that the prefrontally connected portion of the thalamus plays an important role in maintaining high the synchronization between cortical regions whose connectivity may as a result be reduced by the sedation. We found that the thalamus may be part of a network (including the cingulate cortex and a portion of brainstem), which coordinates the different stages of the descent to an unconscious state. The method we used to investigate cortical functional connectivity suggests that the temporal cortex is more sensitive to local connectivity variations, than other regions reported in previous studies [5]. Our results and those from other studies must be seen together, in order to define a model to explain the reversible pharmacological manipulation of consciousness.

Figure *1) Four macroscopic regions of interest were chosen as seeds for the functional connectivity analysis (Frontal, Parietal, Temporal Occipital), by thresholding to 0.7 the related probability MNI structural maps; 2) The results of the paired t-test post>pre and pre>post are reported: Z statistic images were thresholded using clusters determined by Z>2.3 and a (corrected) cluster significance threshold of p<0.05.*

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