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Propofol anesthesia reduces Lempel-Ziv complexity of spontaneous brain activity in rats

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Abstract

Consciousness is thought to scale with brain complexity, and it may be diminished in anesthesia. Lempel-Ziv complexity (LZC) of field potentials has been shown to be a promising measure of the level of consciousness in anesthetized human subjects, neurological patients, and across the sleep-wake states in rats. Whether this relationship holds for intrinsic networks obtained by functional brain imaging has not been tested. To fill this gap of knowledge, we estimated LZC from large-scale dynamic analysis of functional magnetic resonance images (fMRI) in conscious sedated and unconscious anesthetized rats. Blood oxygen dependent (BOLD) signals were obtained from 30-min whole-brain resting-state scans while the anesthetic propofol was infused intravenously at constant infusion rates of 20 mg/kg/h (conscious sedated) and 40 mg/kg/h (unconscious). Dynamic brain networks were defined at voxel level by sliding window analysis of regional homogeneity (ReHo) of the BOLD signal. From scans performed at low to high propofol dose, the LZC was significantly reduced by 110%. The results suggest that the difference in LZC between conscious sedated and anesthetized unconscious subjects is conserved in rats and this effect is detectable in large-scale brain network obtained from fMRI.

1. Introduction

An objective measure of the state or level of consciousness in states of sleep, anesthesia and neurological patients has been a major focus of many investigations. Brain complexity has been postulated as a necessary condition for the conscious state across all phylogenetic and ontogenetic levels [11,16].

Complexity of the electroencephalogram (EEG) has been previously explored as a promising measure of depth of hypnosis and sedation by anesthetic agents [13]. In a recent study,

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transcranial magnetic stimulation (TMS) and algorithmic (Lempel-Ziv) complexity was combined to discriminate the states of consciousness during wakefulness, sleep, and anesthesia in healthy subjects and in patients who had emerged of from coma [4]. A robust decrease in various complexity measures including Lempel-Ziv complexity (LZC) of spontaneous EEG during propofol anesthesia has also been demonstrated [14]. In animal experiments, the LZC of cortical local field potentials showed systematic differences between awake and sleeping laboratory rats and in those undergoing sleep deprivation [1].

As an alternative to electrocortical complexity, functional magnetic resonance (fMRI) signals offer a rich spatiotemporal repertoire that may be used to characterize state-dependent changes of brain complexity. To-date only one study has examined the algorithmic complexity of BOLD signal patterns. Boly et al. showed that LZC of fMRI images correlated with the overall meaningfulness of movie stimuli that were intact vs. scrambled or replaced by television noise [3]. The effect of anesthesia on the complexity of fMRI images has not been investigated.

We recently showed that raising the dose of the anesthetic propofol to a level that induces unconsciousness decreased the temporal variance of fMRI BOLD signal patterns in rats [7]; a similar result was obtained in anesthetized monkeys [2]. This change was interpreted as a reduction in the dynamic repertoire of brain states – a postulated condition of consciousness [5,15]. It is yet unclear if a corresponding change in anesthetic depth is accompanied by a reduction in brain complexity as derived from fMRI data.

To clarify the latter question, in this work we reanalyzed previously acquired fMRI data in six rats obtained at two doses of propofol corresponding to conscious sedation and unconsciousness. As before, we defined a subset or network of brain regions whose BOLD signals exhibited significant spatial correlation during the time course of measurement using the index Regional Homogeneity (ReHo). We then determined the LZC of this network and compared the resulting values between the conscious sedated and unconscious states.

2. Materials and methods

Experimental procedures and protocols were approved by the Institutional Animal Care and Use Committee of the Medical College of Wisconsin (Milwaukee, Wisconsin). All procedures conformed to the Guiding Principles in the Care and Use of Animals of the American Physiologic Society and were in accordance with the Guide for the Care and Use of Laboratory Animals (National Academy Press, Washington, DC, 1996).

The detailed experimental protocol has been published [7]. Briefly, 6 adult, male, Sprague-Dawley rats were scanned during light and deep sedation with propofol at 20 or 40 mg/kg/h equivalent to conscious sedation and unconsciousness [10,12]; the order of doses was reversed in consecutive experiments. The animals' lungs were artificially ventilated; arterial blood pressure, heart rate, arterial oxygen saturation, core temperature, respiratory rate, inspired and expired oxygen and carbon-dioxide concentrations were continuously monitored. The physiological parameters including arterial CO₂ were not different across the range of applied propofol doses [10]. To aid mechanical ventilation, the muscle relaxant

pancuronium bromide (1 mg/kg/h, iv) was added to propofol [7]. To assess the state of consciousness, behavioral sensory responsiveness was tested on the bench in a separate group of animals that did not receive muscle relaxant [10].

The fMRI data were acquired with Bruker 9.4 T AVANCE scanner, Bruker linear transmit coil (T10325) and rodent surface-receiving coil (T9208). Thirty-minute uninterrupted functional scans in the sagittal plane were acquired using a single-shot gradient echo-planar imaging (EPI) sequence: TR = 1000 ms, TE = 19.5 ms, 1800 repetitions, matrix size = 96×96 with the same above geometry, x-y voxel size = $365 \times 365 \text{ } \mu\text{m}^2$. BOLD signal time courses were detrended and low-pass filtered at 0.25 Hz.

Analysis of brain complexity was carried out on a stable, intrinsically connected region of interest defined by the dynamic analysis of regional homogeneity (ReHo). ReHo values of all voxels were calculated using 27 nearest neighbors [17] from 200-s sliding windows with 90% overlap yielding 81 sequential ReHo maps. In each experiment, ReHo values from the low and high propofol doses were concatenated and normalized to SD. From all sliding windows, a common subset of voxels with ReHo values exceeding the threshold of mean + 2 standard deviations (SD) was defined as the region of interest. This threshold was previously found optimal to delineate functionally connected regions of the brain with all voxels present at both anesthetic concentrations [10,12].

To calculate LZC, the ReHo values were mean-subtracted at each time point in each animal and the resulting maps were binarized at a threshold of 1.3 SD within the region of interest. This choice gave a good overall balance of voxels above and below threshold. For a comparison, we also tested the method at 1.0 SD. The result for each experiment and condition was a binary matrix in which rows represent voxels and column represent time points. As introduced by Lempel and Ziv [9], LZC approximates the amount of non-redundant information contained in a string by estimating the minimal number of character sequences or “words” required to describe the string. For a binary sequence, the algorithm [8] searches subsequences of consecutive characters, or “words”. LZC is the count the number of times a new word is encountered. The algorithm by Casali et al. [4] searches a binary matrix for words column by column and normalizes LZC by the source entropy and a factor that depends on matrix size. In this work, LZC was not normalized. The effects of propofol dose on ReHo and LZC were tested using repeated measures ANOVA with rat as subject and dose as within factor using the statistics software NCSS 2007 (NCSS, Kaysville, UT).

3. Results

To illustrate the overall dynamics of ReHo in each experiment, ReHo values from all voxels within the region of interest were averaged. Fig. 1 shows the time course of ReHo averaged across all selected voxels for six experiments for low propofol (conscious sedated) and high propofol (unconscious) states. There was no significant difference in the average ReHo values at 0.64 ± 0.06 vs. 0.71 ± 0.11 at low dose and high dose, respectively ($p = 0.081$, DF = 12, N = 6). Next, the individual ReHo values at were binarized to yield the voxel-time plots as shown in Fig. 2. A difference in the image patterns between low and high propofol

doses is visually evident in all experiments. The calculated LZC values (Fig. 3) confirmed this difference with group average values of 1001(250) at low dose vs. 481(275) at high dose (mean and SD, $p = 0.00055$, $DF = 12$, $N = 6$) implying a substantial and significant reduction in complexity of the unconscious brain. To check the robustness of the method, we also used a lower ReHo threshold of 1.0 SD. The qualitative difference in LZC between low and high propofol doses remained the same in each animal and it was statistically significant ($p = 0.0183$, $DF = 12$, $N = 6$), although the relative difference was smaller at $31 \pm 9\%$ with 1.0 SD vs. $53 \pm 11\%$ with 1.3 SD.

4. Discussion

The present result lends further support to the hypothesis that the conscious brain has higher functional complexity than the unconscious (anesthetized) brain. It supplements the result from investigations in human subjects showing that perturbation complexity index, which is also based on LZC, is able to reliably distinguish the state of consciousness in healthy individuals during wakefulness, sleep, and anesthesia, as well as in patients who had emerged from coma or recovered a minimal level of consciousness [4]. In the latter study, LZC was based on high-density EEG data following perturbation with transcranial magnetic stimulation, whereas our derivation used resting state BOLD activity; nevertheless, the results were highly consistent. Similarly, in rodents, LZC calculated from local field potential activity was higher in wakefulness and rapid eye movement sleep than in non-rapid eye movement sleep especially after sleep deprivation [1]. Our results then supplement these findings with complexity data derived from fMRI BOLD signals for the first time as compared between conscious sedated and unconscious states. Notably, the use of LZC does not require exogenous perturbation such as TMS, which would make it more convenient to translate to a clinical monitor of consciousness during anesthesia. LZC was previously used to compare the tissue-specific determinism of resting-state BOLD data in gray versus white matter and CSF [6].

The present results are also consistent with prior work that revealed a substantial reduction in the temporal variance – a measure of the brain's state repertoire, during propofol-induced unconsciousness in rats and primates [2,7]. Part of our former analysis was also based on ReHo. ReHo delineates a compact 3-dimensional brain region with correlated intrinsic BOLD activity; thus reflecting a functionally connected network of the brain. As calculated, LZC is a measure of the spatiotemporal dynamics of this network.

The results were robust, as the effect of propofol on LZC was qualitatively similar at different SD thresholds used to binarize ReHo. The larger effect of propofol obtained with a higher threshold was probably due to the improved signal to noise ratio capturing physiological BOLD correlations of neuronal activity more than those of fMRI noise.

Finally, we recall that our definition of the conscious state was based on the high sensory behavioral response scores and the desynchronized EEG resembling the awake pattern [10]. Although it cannot be objectively ascertained, these observations imply that in the presence of propofol at a low sedative dose, subjective sensory experience may have occurred consistent with the classification of state as conscious.

In conclusion, the present findings show that the close correlation between LZC and the state of consciousness as modulated by anesthesia is conserved in the human and the rat and emphasize the translational significance of this complexity measure for future animal models in studying the neural correlates of consciousness and unconsciousness.

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HIGHLIGHTS

- Consciousness may scale with brain complexity and may be diminished in anesthesia.
- Lempel-Ziv complexity was estimated from functional magnetic resonance images in rats.
- Complexity of spontaneous brain activity decreased when consciousness was suppressed.

Fig. 1. Time course of regional homogeneity (ReHo) in six rats at low-dose propofol (conscious sedated) and high-dose propofol (unconscious) states.

Fig. 2.

Binary maps of regional homogeneity (ReHo) values thresholded at 1.3 standard deviation from the mean within the region of interest in two consciousness conditions.

Fig. 3. Summary of Lempel-Ziv complexity (LZC) values in conscious sedated and unconscious states in six rats. Between-group difference is significant at $p = 0.00055$.