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Fractal analysis of spontaneous fluctuations of the BOLD signal in rat brain

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ABSTRACT

Analysis of task-evoked fMRI data ignores low frequency fluctuations (LFF) of the resting-state the BOLD signal, yet LFF of the spontaneous BOLD signal is crucial for analysis of resting-state connectivity maps. We characterized the LFF of resting-state BOLD signal at 11.7T in α-chloralose and domitor anesthetized rat brain and modeled the spontaneous signal as a scale-free (i.e., fractal) distribution of amplitude power ($|A|^2$) across a frequency range (f) compatible with an $|A(f)|^2 \propto 1/f^{\beta}$ model where β is the scaling exponent (or spectral index). We compared β values from somatosensory forelimb area (S1FL), cingulate cortex (CG), and caudate putamen (CPu). With α-chloralose, S1FL and CG β values dropped from ~0.7 at in vivo to ~0.1 at post mortem ($p<0.0002$), whereas CPu β values dropped from ~0.3 at in vivo to ~0.1 at post mortem ($p<0.002$). With domitor, cortical (S1FL, CG) β values were slightly higher than with α-chloralose, while subcortical (CPu) β values were similar with α-chloralose. Although cortical and subcortical β values with both anesthetics were significantly different in vivo ($p<0.002$), at post mortem β values in these regions were not significantly different and approached zero (i.e., range of −0.1 to 0.2). Since a water phantom devoid of susceptibility gradients had a β value of zero (i.e., random), we conclude that deoxyhemoglobin present in voxels post-sacrifice still impacts tissue water diffusion. These results suggest that in the anesthetized rat brain the LFF of BOLD signal at 11.7T follow a general $1/f^{\beta}$ model of fractality where β is a variable responding to physiology. We describe typical experimental pitfalls which may elude detection of fractality in the resting-state BOLD signal.

Introduction

Similar to earlier observations in various physiological systems (Bassingthwaighte et al., 1994; Buzsaki and Draguhn, 2004; Dora and Kovach, 1981; Eke et al., 2002, 2006; Gilden et al., 1995; Hausdorff et al., 1997; Makikallio et al., 2001; Obrig et al., 2000), the blood-oxygenation level dependent (BOLD) signal in the brain, obtained non-invasively by functional magnetic resonance imaging (fMRI), shows spontaneous low frequency fluctuations (LFF) at rest that cannot be attributed to response to external stimuli (Fox and Raichle, 2007). While LFF of the resting-state BOLD signal is inherently a temporal phenomenon, its significance was first recognized in the spatial domain in the form of cross-correlation maps (Biswal et al., 1995). It was only later, that the LFF of the resting-state BOLD signal was demonstrated to follow a "1/*f distribution*" (i.e. "1/*f* noise") in the frequency domain (f), suggesting that there could be a systematic increase in the amplitude power ($|A|^2$) across the low frequencies (Zarahn et al., 1997) otherwise known as "inverse power–law scaling" that can be demonstrated by fitting a spectral slope across the power estimates (Eke et al., 2002).

The origin of the "1/*f* noise" or "inverse power–law scaling" (Eke et al., 2002) in the LFF of the resting-state BOLD signal is still unclear. One may consider physiological and technical contributing factors. As to the physiological factor, the BOLD signal may be influenced by hemodynamic and metabolic factors (cerebral blood flow or volume and cerebral metabolic rate of oxygen, respectively) as well as neural activity itself (Hyder et al., 2001; Ogawa et al., 1993). Studies assessing fluctuations of blood flow in the rat cerebral cortex by laser-Doppler flowmetry (LDF) and oscillations of resting membrane potential (V_m) in the cat cerebral cortex with electrophysiology have demonstrated that these physiological signals exhibit inverse power–law scaling in the frequency domain of the "$1/f^{\beta}$" type, where the spectral slope or its negative value, the power spectral scaling exponent, β, was found to deviate from −1 or 1, respectively (Eke et al., 2000, 2002; El Boustani et al., 2009). As to the technical factors, a spectrum of the LFF of the resting-state BOLD signal could be contaminated by system noise introduced by the fMRI scanner

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generating a spatially anisotropic 1/*f*-like noise in the magnet bore subject to the actual distance of the probed voxel from the isocenter.

As a result of rapid developments in the field, it is recognized that the inherently complex functioning of the brain represented by neural activities and associated hemodynamic and metabolic responses, presumably captured by the LFF of the BOLD signal, can be described and analyzed by different paradigms such as fractality, self-organized criticality, or modularity (Bullmore et al., 2009). Fractality is to detect the presence of power–law scaling frequency distribution according to the formalism of the "$1/f^{\beta}$" model which characterizes the temporal aspects of the complexity of the brain independently of the modality of the signal (Eke et al., 2000). Most notably, blood flow fluctuations as measured by LDF and neural activity fluctuations measured by electrophysiology (El Boustani et al., 2009; Herman and Eke, 2006) as well as the LFF of the resting-state BOLD signal may follow this general fractal model as earlier demonstrated in the human brain by Thurner et al.(2003). These authors showed that voxel-wise temporal distribution of spontaneous fluctuations of the resting-state BOLD signal did not follow a "$1/f$" model as the spectral slope varied according to the functional-metabolic activity of the neuronal tissue within the region of interest (ROI) (Thurner et al., 2003). Such variations can only be accounted for by the general "$1/f^{\beta}$" model (Eke et al., 2000, 2002). Later, Maxim et al. performed scale-free analysis of the LFF of the resting-state BOLD signal and reported that another scaling parameter—the Hurst exponent, H—was found to correlate with altered mental state such as Alzheimer's disease (Maxim et al., 2005). Since then, others reported H values of about 0.3 to 0.6 for the resting-state gray matter LFF of the BOLD signal in human and rat brain (He et al., 2010; Wang et al., 2011; Wink et al., 2006, 2008). A critical evaluation of these studies reporting various fractal measures (i.e. β and H) would become only possible if the implications of the "$1/f^{\beta}$" model as it relates to the signal character pertinent to the dichotomous fractal process model of Mandelbrot and Van Ness (Eke et al., 2000; Mandelbrot and van Ness, 1968) was fully appreciated. Specifically, based on β two signal categories of the dichotomous fractal process model of Mandelbrot and Van Ness can be defined (Eke et al., 2000; Mandelbrot and van Ness, 1968). With $\beta>1$ the signal qualifies as fractional Brownian motion (fBm, a non-stationary process with variance dependent on time), and with $\beta<1$ as fractional Gaussian noise (fGn, a stationary process with variance independent of time). Note that $\beta=1$ ($1/f$ noise) and $\beta=0$ (white noise) are merely two special cases within these families of signals (Eke et al., 2000, 2002). The relationship between β and H is complex: for fGn and fBm signals $H=(\beta+1)/2$ and $H=(\beta-1)/2$, respectively (see Appendix A for further fGn and fBm subcategories).

Our aims in this study were to test the following hypotheses: i) the LFF of the BOLD signal from anesthetized brain follows the general $1/f^{\beta}$ model of fractality with a variable scaling exponent; ii) the $1/f^{\beta}$ fractal structuring is a manifestation of physiological processes and not of artifactual fluctuations due to fMRI scanner noise (e.g., gradient noise). To test the first hypothesis, we obtained resting-state BOLD signals under deep and light general anesthesia (i.e., α-chloralose and domitor, respectively) and performed spectral analysis of fractality. To test the second hypothesis, we removed all physiological contributions to the BOLD signal by making measurements post mortem and in a phantom).

Methods

Animal preparation

All procedures were performed according to protocols approved by the Ethical Committee of Yale University School of Medicine and the Institutional Animal Care and Use Committee and in agreement with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All experiments were conducted on adult male rats ($n=7$; Sprague–Dawley; 200–300 g; Charles River, Wilmington, MA) tracheotomized, artificially ventilated and anesthetized with 1–2% halothane or isoflurane during surgery (70% N_2O and 30% O_2). After surgery, anesthesia was switched to α-chloralose (~40 mg/kg/h; i.p.) which provides deep anesthesia with low global brain energy metabolism (Maandag et al., 2007). In addition, we also used domitor (0.1 mg/kg/h, i.p.; $n=4$) instead of α-chloralose, which is known to provide lighter anesthesia and higher brain energy metabolism (unpublished results, PH, BGS, FH). Muscle relaxant (*D*-tubocurarine chloride, ~0.3 mg/kg/hour; i.p.) was used to provide immobilization during the fMRI scans with regular checking on pain reflexes (i.e. electrical tail pinch reflex). The femoral artery and vein were cannulated for physiological monitoring and possible infusion of drugs. The arterial blood pressure, intra-alveolar pressure, and core body temperature were monitored continuously and every *in vivo* fMRI image was labeled with reference to these measurements. Blood gas parameters (pCO_2, pO_2, pH) were measured periodically. The animal was covered with a water heated blanket to maintain core temperature at 37 °C. The animal was placed at the magnet isocenter for all resting-state fMRI recordings. Following the in vivo scans under α-chloralose anesthesia, we gave a high dose (5%) of isoflurane for 10 min and euthanized the animal with concentrated KCl intravenous infusion while maintaining isoflurane. The animal remained in the scanner for an hour while repeated post mortem fMRI scans were performed.

fMRI studies

All fMRI data were obtained by a modified 11.7T Bruker horizontal-bore spectrometer (Bruker AVANCE, Billerica, MA) using a ^1H surface coil (1.4 cm diameter). Shimming was optimized with adjustment of 1st and 2nd order shims (Gruetter, 1993). All fMRI data were collected with sequential sampling gradient echo planar imaging (EPI) sequence (Hyder et al., 1995): field of view of 2.56×2.56 cm^2; image matrix of 64×64; slice thickness of 2 mm; repetition time of 200 ms (i.e., 5 Hz of sampling frequency), and echo time of 13 ms; and voxel size of $400\times400\times2000$ μm^3. 32 dummy scans were carried out before fMRI data acquisition began. We acquired 4200 images of which only 4096 images (2^{12}) were used thus creating BOLD time series in adequate length for fractal analysis using the EPI sequence (Eke et al., 2000). Neuroanatomy was imaged with either RARE (Hennig et al., 1986) or FLASH (Frahm et al., 1986) pulse sequences.

All fMRI data were subjected to a translational movement criterion using a center-of-mass analysis (Chahboune et al., 2007). For each series, two center-of-mass values were calculated, one for each in-plane direction. If either center-of-mass value in a series deviated by more than ¼ of a pixel, the entire dataset was discarded from further analysis. The image series were manually masked to differentiate brain and non-brain voxels. Furthermore, only those voxels having a signal-to-noise ratio (SNR) of higher than 30 dB were used in the analysis. SNR was calculated for every voxel as $20*\log_{10}(\text{mean/SD})$, where SD is the standard deviation of the signal over elapsed time (>200 s).

The in vivo EPI data were collected in steady-state within 15 min after the animals had been stabilized in the scanner. The post mortem EPI data were collected after 1 h following the sacrifice of the animal. All EPI parameters were the same for in vivo and post mortem data acquisitions. The phantom data were collected with parameters identical to those used in brain data acquisitions. The phantom data served as reference for post mortem data analysis. The phantom contained 0.9% NaCl solution in a mixture of 90% D_2O and 10% H_2O. Similar to brain data acquisition, it was placed at the magnet isocenter for data acquisitions.

Pre-processing of data

The voxel-based time series of the BOLD signal were created after quality control assessment (i.e., SNR of each data set). The 4096 data