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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.

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Keywords

resting-state connectivity; BOLD signal; fractality; self-similarity; scale-free correlation; frequency domain analysis; non-linear dynamics

1. INTRODUCTION

Similar to earlier observations in various physiological systems (Bassingthwaight et al., 1994; Buzsaki and Draguhn, 2004; Dora and Kovach, 1981; Eke et al., 2006; Eke et al., 2002; 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; Eke et al., 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 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.* (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) (Turner et al., 2003). Such variations can only be accounted for by the general “ $1/f^\beta$ ” model (Eke et al., 2000; Eke et al., 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; Wink et al., 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; Eke et al., 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 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).

2. METHODS

2.1. 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% N₂O and 30% O₂). After surgery, anesthesia was switched to α -chloralose (~40 mg/kg/hour; 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/hour, 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 (pCO₂, pO₂, 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 minutes 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.

2.2. 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 $2.56 \times 2.56 \text{ cm}^2$; 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 $400 \times 400 \times 2000 \mu\text{m}^3$. 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 $\frac{1}{4}$ 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 $20 \cdot \log_{10}(\text{mean}/\text{SD})$, where SD is the standard deviation of the signal over elapsed time ($> 200 \text{ s}$).

The in vivo EPI data were collected in steady-state within 15 minutes after the animals had been stabilized in the scanner. The post mortem EPI data were collected after one hour 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.

2.2.1. 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 points were collected at 5 Hz sampling rate (Figure 1A) such that the frequency analysis revealed the power spectrum between 0.001 and 2.5 Hz (Figure 1B), the Nyquist frequency which is half the sampling frequency. The double logarithmic representation of the spectrum revealed that the frequency distribution could be divided into three characteristic segments according to their local spectral slopes (Figure 1B). The boundaries of these segments – represented as inflection points between adjacent spectral estimates with apparently different local slopes – varied very slightly from data in different voxels. Thus instead of defining the boundary frequency limits for each and every time series, we selected the frequency range of 0.02–0.3 Hz because power-law scaling behavior was ubiquitous and common for all time series in our data bank in this range of frequencies (Figure 1C). Since our intention was to investigate the power-law scaling behavior of the resting-state BOLD signal, we removed the frequency components below 0.02 Hz and above 0.3 Hz and used only the middle range of the captured dynamics for analysis (Figure 1D).

Peaks due to synchronized vasomotion, otherwise known as the Traube-Hering-Mayer wave, can typically emerge at around 0.1 Hz (Mayer, 1876). If this vasomotion peak is not removed, the fractal analysis may be compromised. The procedure of the peak removal

consisted of the following steps (Supplementary Figure 1). An actual segment between 0.075 and 0.16 Hz of the spectrum confining the peak was selected and a mask was created for an automatic elimination of the peak. Power estimates within the following two frequency ranges were processed: one in the vicinity of the peak (within the mask) and another outside the mask. The spectral estimates within these two ranges were least squared fitted to a Gaussian distribution and a regression slope, respectively, while in an iterative process convergence on a minimal degree of deviation between raw and fitted estimates for both of these ranges was achieved as the range confining the peak was being narrowed to optimal. Upon convergence, the power slope was found for the estimates outside the Gaussian template and was used to calculate β (see below), now free from the bias of local power peak, if present. Application of this peak removal to data series actually lacking this peak did not affect results of their fractal analysis (Supplementary Figure 2).

2.2.2. Selection of cortical and subcortical ROIs—All fMRI data were co-registered to the rat brain atlas and were analyzed for three ROIs of somatosensory forelimb area (S1FL), cingulate cortex (CG), and caudate putamen (CPu) as identified in rat brain atlas (Paxinos, 1996). The S1FL and CG were of similar size, whereas CPu was about 3-4 times larger.

2.3. Statistical analyses

Mean and SD of each time series were used for statistical characterization of the results. Statistical evaluations were carried out in Statistica 8 (StatSoft, Inc. Tulsa, OK, USA). Significance between groups was reported with actual p values obtained by repeated measures of ANOVA using Newman-Keuls post-hoc test. In addition, by the use of power analysis we confirmed that the number of animals in this study was successfully minimized – as requested by Yale University regulations – while assuring that this number would still provide a statistically representative sample for analysis.

2.4. Scale-free spectral analysis of fractality

Subsequent to pre-processing filtering, fractal properties in resting-state BOLD time series were evaluated according to the numerically tested power spectral density (PSD) method of Eke *et al.* (Eke et al., 2000). An FFT produces the amplitude (A), phase (ω), and power ($|A|^2$) of the signal, respectively, for the different frequency components (f). The PSD of a fractal process is of the form of a power-law relationship if $|A(f)|^2 \propto 1/f^\beta$, where β is termed as the spectral index and which in turn is the negative slope of the spectrum on its log-log plot. The power-law relationship – as a fundamental property of scale-free time series – expresses the idea that as one moves along the frequency scale, the power will change by the same fraction (β) regardless of the chosen leap along the frequency scale, i.e., the ratio is independent of where one is on the frequency scale. The power-law relationship thus captures a scale-free property (slope) of the spectrum and demonstrates the presence of self-similarity in the frequency domain (or better yet, self-affinity given that the scaling along the two axes of the spectrum – power and frequency – are different). The signal is preprocessed before the FFT by subtracting the mean, multiplying with a parabolic window or windowing and bridge detrending or endmatching (Eke et al., 2000; Fougere, 1985).

3. RESULTS

3.1. Signal characterization and fractal modeling

The spontaneous LFF of rat brain BOLD signal were characterized in vivo and post mortem (Figure 2) in the frequency domain as $|A(f)|^2 \propto 1/f^\beta$, where β is the scaling exponent and is given by the negative of the slopes in the log-log frequency domain plots in the lower panel of Figure 2A and Figure 2B. The time series shown are from two randomly selected animals.

For a similar voxel placed in the S1FL of two rats, the value of β for in vivo data shown are 0.86 and 1.29 and for post mortem are 0.03 and 0.02, which suggest that the in vivo data have fractal properties, whereas the post mortem data do not. The goodness-of-fit of $1/f^\beta$ model for the selected range of frequency was characterized by the R^2 value (0.81 ± 0.11 for ~ 9000 model fit)

3.2. Impact of synchronized vasomotion and arterial blood pressure fluctuations

Since an isolated vasomotion peak at ~ 0.1 Hz can obviously introduce bias in estimating β when fitting the linear trendline across the spectral estimates of the spectrum, this peak was eliminated (by decomposition) prior to further analysis. This isolated vasomotion peak is however a rare finding (Hudetz et al., 1995). In fact, its incidence in our study was less than 2% of the total ~ 9000 time series we studied (from 7 rat brains). Its distribution in the cerebral cortex was heterogeneous with a varied localization from animal to animal. Estimation of β for voxels without a synchronized vasomotion power peak in their spectra was unaffected by the automated procedure of peak removal (Supplementary Figure 2). Moreover, fluctuations in BOLD signals were independent of changes in the arterial blood pressure (Supplementary Figure 3).

3.3. Impact of signal length and sampling rate

For bias-free fractal analysis it is crucial to acquire data in the appropriate length and sampling rate (Figure 3) as we tested earlier with extensive numerical simulations (Eke et al., 2000). Accordingly, in this study each time series of resting-state BOLD signal fluctuations was of sufficient length ($n = 4096$ data points) and high enough sampling rate (5 Hz) to provide an accurate representation of the frequency structure embedded within the spontaneously fluctuating signal. The length of the data series determines the increments between available neighboring frequencies and together with the sampling rate it determines the smallest frequency component in the spectrum (Figure 3A). The importance of using relatively high sampling rate is to avoid artificial frequency components being aliased across the spectrum (Figure 3B). In other words, a high sampling rate is needed to identify wide bandwidth contributions. As for the length of data series, the longer the time series the better the lowest frequencies are captured in the spectrum (Eke et al., 2002). It should be noted, however, that a low sampling rate tends to misrepresent the fractal structure of the resting-state fMRI data set much more than a shorter time series does (e.g., see low panels of Figure 3B).

3.4. Topology of fractally fluctuating resting-state BOLD signals

The in vivo spatial distribution of β was found topographically characteristic in that voxels corresponding to cortical areas exhibited higher values than those in subcortical areas (Figure 4). The β maps obtained from anesthetized rat brain were found stable over time (Figure 4A) as assessed by differencing individual β maps acquired over different time periods from the mean β map for a given rat (Figure 4B). The reproducibility of these maps from different animals is shown in Figure 4C. While cortical and subcortical regions could be distinguished by β values in vivo, this pattern was diminished at post mortem (Supplementary Figure 4). We compared β values in two cortical (S1FL, CG) and one subcortical (CPu) ROIs (Figure 5A) to find that β values for in vivo and post mortem were significantly different: S1FL (0.74 ± 0.17 vs. 0.17 ± 0.06 , $p < 0.0001$), CG (0.67 ± 0.21 vs. 0.09 ± 0.05 , $p < 0.0002$), CPu (0.30 ± 0.08 vs. 0.12 ± 0.09 , $p < 0.002$) (Figure 5B). It should be noted, however, that cortical and subcortical regions were significantly different in vivo (S1FL vs. CPu with $p < 0.0002$; CG vs. CPu with $p < 0.002$), but not in post mortem (S1FL vs. CPu with $p > 0.23$; CG vs. CPu with $p > 0.48$). Specifically, in vivo the β distributions of S1FL and CG are greatly overlapping with CPu with a β range of 0.3 to 0.8 (Figure 5C), whereas in post mortem all regions are nearly fully overlapping at β values ranging from -0.1 to 0.2

(Figure 5D), which is indeed close to the random category (white noise) for β and as seen in the phantom data (Supplementary Figure 4). Both the in vivo and post mortem patterns persisted within a narrow range of scatter, indicating that the anesthetized brain did not spontaneously change its spatial autocorrelation pattern (see Discussion).

The domitor anesthetized animals generally showed similar topology of β values (Figure 6A) as observed with the α -chloralose anesthetized animals (Figure 4C) - i.e., higher values in vivo in the cortex (S1FL,CG) and lower in subcortex (CPu) – but all values significantly decreased post mortem close to zero value (Figure 6B): S1FL (0.97 ± 0.21 vs. 0.11 ± 0.15 , $p < 0.001$), CG (1.18 ± 0.23 vs. 0.09 ± 0.14 , $p < 0.001$), CPu (0.31 ± 0.21 vs. 0.05 ± 0.14 , $p < 0.001$).

Given that all fMRI data are suspect to SNR issues, we investigated the possibility that SNR variations across brain regions may have accounted for the cortical and subcortical differences observed in the β maps. Similar to the mean β values in the three ROIs and the β histograms for in vivo and post mortem, we plotted the mean SNR values in the three ROIs (Supplementary Figure 5A) and the SNR histograms for in vivo (Supplementary Figure 5B) and post mortem (Supplementary Figure 5C). SNR difference between in vivo and post mortem were not significant and SNR distributions of S1FL, CG, and CPu were largely overlapping. However there was a slight drop in SNR post mortem. In vivo and post mortem SNR for CPu was smaller than in S1FL and CG. These results suggest that slight SNR variations across the ROIs could not account for the significant differences in β values observed. In fact, there were no correlations found between β values and SNR in vivo (Supplementary Figure 6A) or post mortem (Supplementary Figure 6B).

4. DISCUSSION

Studying the significance of scale-free spontaneous fluctuations in functional parameters of the brain has emerged as a new powerful paradigm with the premise being that what earlier seemed mere random noise, and accordingly dealt with by conventional descriptive statistics, may now be regarded as a source of valuable information on the complex inner workings of the brain. Results of the present study demonstrate that the in vivo LFF of the resting-state BOLD signal shows scale-free fluctuations, a fundamental property of fractality. Scale-free because the power-law relationship – which models the signal as observed in the frequency domain (Figure 1) – expresses the idea that as one doubles the frequency, the power diminishes by the same fraction regardless of the chosen frequency – i.e., the ratio is independent of where one is on the frequency scale (Eke et al., 2000). Equivalent terminologies for scale-free are “self-similar” or “fractal” (Eke et al., 2002). In this study, we characterized the LFF of resting-state BOLD signal at 11.7T in anesthetized rat brain and modeled the spontaneous signal as $|A(f)|^2 \propto 1/f^\beta$, where β is the scaling exponent (Figure 2). We also tested the special $1/f$ model (i.e., where β is 1) as follows. As seen in the histogram plot of β values in Figure 5, this model would be adequate only if the relative frequencies were peaked with a narrow scatter around $\beta = 1$. This is clearly not the case given the broad range of distribution of β values. In consideration of all in vivo data, we found that under no circumstance was $\beta = 1$ for CPu, whereas for S1FL and CG only 7.8 % of the population fell in the range of $0.95 < \beta < 1.05$. On average, we found higher β values in the cortex than in the subcortex in the anesthetized brain (Figures 3 and 4). This difference diminished post mortem when β values in these structures ended up scattering around the random value of $\beta = 0$ (Figures 5 and 6). The results suggest that LFF of BOLD signal at 11.7T in rat brain follow a general $1/f^\beta$ model of power distribution, where β is a physiological variable.

4.1. Prerequisites for reliable fractal time series analysis of the BOLD signal

4.1.1. Importance of signal length and sampling rate—The essence of fractal time series analysis of LFF in the BOLD signal is to properly assess and quantify the scale-free behavior by calculating β (or for that matter by calculating H , the Hurst exponent), both being scaling exponents in the frequency and time domains, respectively (Eke et al., 2002). This can only be achieved by acquiring fMRI data in adequate data length and at high enough sampling rate (Figure 3). In contrast with recent human and animal resting-state fMRI studies utilizing scale-free analysis (He et al., 2010; Maxim et al., 2005; Thurner et al., 2003; Wang et al., 2011; Wink et al., 2006; Wink et al., 2008), our rat brain fMRI data sets are of much higher temporal resolution to provide a wider bandwidth that allows better definition of the BOLD signal in the low frequency range which is an essential prerequisite for reliable fractal analysis of time series data (Eke et al., 2000; Eke et al., 2002). One may use mathematical methods to demonstrate the impact of signal length and sampling rate on the precision of fractal estimates (such as β or H) of scale-free fluctuations in numerically simulated time series data (Eke et al., 2000; Eke et al., 2002). In this study, however, we provide a visual demonstration to the same effect using real fMRI time series data (Figure 3), emphasizing these important prerequisites (i.e., appropriate length and sampling frequency) of a reliable fractal analysis of LFF of the resting-state BOLD signal.

In our study, we observed that as the acquisition time decreased the difference between β values in cortical vs. subcortical ROIs became more exaggerated (Figure 3A) and as the sampling rate decreased the difference between β values in cortical vs. subcortical ROIs diminished with CG and other deep brain regions emerging with high β values (Figure 3B). A recent rat brain study at 4.7T with isoflurane anesthesia shows values of H in S1FL and CPU to be indifferent, but upon increasing depth of anesthesia values of H decreased from ~ 0.6 to ~ 0.5 which correspond to β values of ~ 0.2 to near-zero (Wang et al., 2011). While the results of the Wang *et al.* showing diminishing β with decreased brain activity are in accord with our findings of decreased β from in vivo to post mortem (e.g., see Figures 2 and 5), there are some technical differences to note between these two studies.

Since the fMRI data acquisition parameters of Wang et al. corresponded to a data length of 600 s at 1 Hz, we compare this situation with our data length of 840 s at 1 Hz. In Figure 3B see left of upper panel for data length of 840 s at 5 Hz and see middle of lower panel for data length of 840 s at 1 Hz. The β map with data length of 840 s at 1 Hz shows (see middle of lower panel in Figure 3B) congruence between values in S1FL and CPU as found in the Wang *et al.* study. However in the optimal case of data acquisition with data length of 840 s at 5 Hz (see left of upper panel in Figure 3B) the very same β map looks significantly different due to the improved dynamic resolution. Thus we believe that appropriate sampling rate (and data length) should be used for fractal analysis of BOLD signal as demonstrated earlier in numerical experiments of fractal time series analyses (Eke et al., 2000). Moreover the difference in magnetic field strength between our study (at 11.7T) and the Wang *et al.* study (at 4.7T) may have contributed to some differences for the regional β values.

4.1.2. Importance of data pre-processing—We identified two physiological factors that may have potentially interfered with our fractal time series analysis: synchronized vasomotion (Supplementary Figure 2) and arterial blood pressure fluctuations (Supplementary Figure 3). While we did observe vasomotion peaks in our BOLD data, this phenomenon was present in less than 2% of the evaluated time series. Furthermore the automated power peak removal procedure (Supplementary Figure 1) did not disrupt the characteristic topology of the β map (Supplementary Figure 2). In other words, time series not having a vasomotion peak were not affected.

We simultaneously measured arterial blood pressure with fMRI in all experiments. Blood pressure is known to oscillate, and in principle, these fluctuations could propagate to the level of the imaged voxels along the supplying arterial tree and thus may influence the voxel-based hemodynamics. However arterial blood pressure itself did not interfere with our fractal analysis given that it was found to be uncorrelated with the resting-state BOLD signal fluctuations (Supplementary Figure 3).

4.2. Fractal characterization of cortical baseline state

The β maps obtained under anesthesia (Figure 4) and post mortem (Supplementary Figure 4) showed stable topology, indicating that the β value could be an indicator of physiological states across the brain. While in vivo cortical and subcortical regions could be readily discerned based on their respective β (S1FL vs. CPu with $p < 0.0002$; CG vs. CPu with $p < 0.002$ under α -chloralose and $p < 0.0001$ with domitor anesthesia), in post mortem the β maps became less articulate over these regions (S1FL vs. CPu with $p > 0.23$; CG vs. CPu with $p > 0.48$ with α -chloralose and $p > 0.33$ with domitor anesthesia) (Figure 5B, Figure 6B). In this study, brain's spontaneous activity level was profoundly altered – from in vivo to post mortem – to define the full scale of β values. Studies are now underway in our laboratory – similar to that of Wang et al. (Wang et al., 2011) – to investigate changes in β value as a function of subtle alterations in baseline state in vivo. We can assert that the level of anesthesia did not interfere with the trend of finding high β values in the brain while alive and ubiquitously low β values at post mortem (Figure 5B, Figure 6B). Moreover, domitor, which induced lighter anesthesia compared to α -chloralose, yielded higher β values in cortical areas, especially in CG, while leaving those in the subcortical regions unaltered.

Histograms representing distributions of β values in vivo for S1FL and CG showed great overlap with each other, but only partial overlap with CPu (Figure 5C). The in vivo β range for cortical and subcortical regions overlapped between ~ 0.3 and ~ 0.8 . Histograms post mortem for S1FL, CG, and CPu showed great overlap with each other peaked at a β value of 0.1, ranging between -0.1 and 0.2 (Figure 5D). Since SNR in principle could have contaminated the β maps, we obtained histograms of SNR as well. Close inspection of the data shows that SNR across these ROIs vary slightly but nearly not sufficiently enough to account for the magnitudes in β values observed either with respect of regions in vivo or in vivo versus post mortem (Supplementary Figure 5). This notion is supported by the fact that no correlation between β values and SNR exists (Supplementary Figure 6). These findings assert that the β values were not contaminated by SNR variations.

Since the marked difference between cortical and subcortical β values cannot be attributed to surface coil properties (as discussed above), we hypothesize that β value variations may have anatomical and/or physiological origins. In support of this rationale, autoradiographic studies of blood flow and metabolism in conjunction with regional capillary density studies show that there are marked differences between cortical and subcortical regions of the rat brain (Borowsky and Collins, 1989; Iadecola et al., 1983). Glucose metabolism, blood flow, and capillary densities are significantly higher in the cerebral cortex compared to many subcortical regions. The fact that α -chloralose and domitor had different impacts on the cortical, but nearly similar ones on the subcortical β values (Figure 5B and 6B) suggests that subcortical BOLD signal fluctuations may not be baseline dependent as previously noted with metabolic changes elicited by different anesthetics (Ueki et al., 1992).

4.3. Impact of fMRI system noise on β

The time-invariant autocorrelation pattern of LFF in the BOLD signal – being the product of in vivo processes – is expected to abolish after death. Indeed we found that nearestneighbor correlation coefficient (r_1), which describes the fractal autocorrelation (see Appendix for

details on fractal autocorrelation), decreased from the in vivo level of 0.67 to 0.12 at post mortem (corresponding to β values of 0.74 and 0.17, respectively) in the cortical S1FL region, and it similarly decreased from 0.61 to 0.06 in CG. The corresponding r_1 values for the subcortex (CPu) are 0.23 and 0.08, indicating a low degree of temporal correlation in vivo and almost no correlation at post mortem. Due to lack of underlying physiology given a commenced state of death, the post mortem β values were expected to be zero with a random correlation structure (white noise with $\beta=0$ and $r_1=0$) meaning equal distribution of spectral estimates across the sampled frequencies and no correlation between the temporal events. However we found that while β did decrease at post mortem from its in vivo level, it actually did not reach zero.

A possible explanation of the non-zero β values at post mortem may be rooted in the origin of the BOLD signal which emerges from paramagnetic susceptibility influences of deoxyhemoglobin and is linked to metabolic activity and its elicited hemodynamic response (Ogawa et al., 1993). The metabolic and hemodynamic factors are obviously absent in the dead brain. However deoxyhemoglobin molecules are still present in the MRI voxels post-sacrifice and thus generate susceptibility-induced magnetic field gradients that will impact diffusion of tissue water molecules, a process which may not be completely random (Hyder et al., 2001). In contrast, a pure water phantom which does not have susceptibility-induced magnetic field gradients does demonstrate β values of zero (i.e., random) using identical fMRI parameters for data acquisition as the in vivo experiments. Another possible explanation could be that the LFF of the BOLD signal may still be contaminated by an artificial $1/f$ -type contribution from the scanner (e.g., shim gradients, spatial gradients, etc.) which can cause fluctuations in the static magnetic field by eddy currents, and consequently in the BOLD signal itself; an effect that could even be spatially varying away from magnet isocenter (Zarahn et al., 1997). Indeed, using a small phantom we found β value of about zero in the isocenter. In principle, β values can be influenced by the distance from the isocenter. However marked bias due to system noise on the reported β values in this study is unlikely. An artificial $1/f$ -type contribution from scanner noise occurs at a very low frequency range (Zarahn et al., 1997). Our fractal analysis of the fluctuations in the BOLD signal was focused within a bandwidth of 0.02 to 0.3 Hz which is well above the reported frequency range of system noise contribution. Furthermore we report a drastic decrease in β value in post mortem. A contribution from scanner noise would have certainly degraded the topology in our reported β maps.

4.4. Impact of physiology on β

A recent study of Razavi *et al.* shows that the hemodynamic component has to be considered as a dominating source for LFF in the resting-state BOLD signal (Razavi et al., 2008). In previous studies we measured LFF in cerebral hemodynamics with optical methods like laser-Doppler flowmetry, laser speckle contrast flow imaging, and near-infrared spectroscopy (Eke and Herman, 1999; Eke et al., 1997; Eke et al., 2000; Eke et al., 2006; Herman and Eke, 2006; Herman et al., 2009a). In spite of the diversity in methods and species, it was a common finding in these studies that resting-state cerebral hemodynamics fluctuated spontaneously according to a scale-free $1/f^\beta$ model, with β as a variable.

But the BOLD signal is a complex phenomenon with components dependent on blood oxygenation, flow, volume, and oxidative metabolism (Herman et al., 2009b; Logothetis and Wandell, 2004; Sanganahalli et al., 2009). In theory, local neuronal activities, metabolic demands, signaling along the neuroglia and vascular endothelium, and microregional perfusion can all be likely contributors to the $1/f^\beta$ process – not only due to their causal relationships and/or interactions between them, but also due to their progressively increasing time scales of operation blending them into a $1/f^\beta$ pattern for LFF in the BOLD signal. In other words, the interactions of hemodynamic, metabolic, and cellular factors may not

contribute in a predictable fashion to the $1/\beta$ process. In support of this suggestion a recent study by Kiviniemi *et al.* shows that hypocapnia-induced reduction of blood flow actually increases the fractal parameter of the BOLD signal (Kiviniemi *et al.*, 2009).

4.5 Future perspectives

It was only recent that fMRI studies in rat brain demonstrated cross-correlated patterns in LFF of the resting-state BOLD signal which are used to generate functional connectivity maps (Pawela *et al.*, 2008; Zhao *et al.*, 2008). In our current study, and as Wang *et al.* showed recently (Wang *et al.*, 2011), the autocorrelated LFF of the resting-state BOLD signal previously observed in humans is also present in rat brain suggesting that fractally autocorrelated LFF of the BOLD signal is a fundamental feature of the mammalian brain.

Scale-free fractal exponents such as β have proven to be of prognostic value in cardiology (Makikallio *et al.*, 2001). Recognizing that β is a variable and then applying proper tools to reliably assess it is a fresh perspective open to both basic and clinical neurosciences (Eke *et al.*, 2002; Hausdorff *et al.*, 1997; Pilgram and Kaplan, 1998). Future studies could evaluate the dependence of β when varying baseline levels (Maandag *et al.*, 2007; Smith *et al.*, 2002) as is commonly done in human fMRI studies (van Eijsden *et al.*, 2009). As demonstrated in this study, the $1/\beta$ model of the brain's BOLD signal fluctuations, shown here in rat brain, ought to be viewed with the possibility that β could be a physiological variable and it could prove to be a biomarker worthy of further study, e.g., distinguishing cortical and subcortical areas by scale-free parameterization of the LFF in the BOLD signal.

We may hypothesize that our findings in the cortex i) identifying a significant population of non-stationary fMRI signal, ii) an inverse relationship of the population size of the non-stationary signals with the depth of anesthesia may have impact on human functional connectivity studies using resting-state fMRI, because non-stationary behavior may yield false results in correlation analysis of resting-state fMRI. Therefore it seems important, and perhaps even necessary, to characterize the fractal properties of the spontaneous BOLD signal, which as shown here, can be achieved with high temporal resolution fMRI. There are recent examples for much improved temporal resolution studies using high field human scanners (Feinberg *et al.*, 2010; Poser *et al.*, 2010). Moreover recent human and primate resting-state fMRI studies have also been conducted under anesthesia (Boveroux *et al.*, 2010; Vincent *et al.*, 2007) and effective connectivities are being assessed between cortex and subcortex as well (Skudlarski *et al.*, 2010; Yu *et al.*, 2011).

Undoubtedly these explorations would be strengthened by simultaneous multi-modal imaging of the brain (Hyder and Rothman, 2010). Another likely direction could be to test the applicability of multi-fractal models in order to reveal an even more refined structuring in spontaneous resting-state BOLD fluctuations, if present, especially in different baseline states (Wink *et al.*, 2008).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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APPENDIX

The spectral index, β , does not only refer to the scale-free (i.e., fractal) behavior of the signal, but to its correlation structure itself otherwise known as autocorrelation (Eke et al., 2002). The extent of autocorrelation is tightly coupled to β . With β between -1 and 0 , the signal is a strongly anticorrelated fractional Gaussian noise (or $-fGn$). Moving in the direction of increasing β values, the signal becomes weakly anticorrelated (or $-fGn$), then it reaches randomness when β is 0 . Similarly, for β values greater than 0 , the signal first becomes a weakly correlated fractional Gaussian noise (or $+fGn$) then with $0.5 < \beta < 1$ a strongly correlated fractional Gaussian noise (or $++fGn$). For β values greater than 1 , the signal is also regarded strongly correlated but in the fractional Brownian motion (or fBm) domain. However due to the peculiarities of the dichotomous fGn/fBm model yielding fBm signals as the cumulative sum of their fGn counterparts offset by $\beta = -2$, the autocorrelation becomes restricted to fGn type signals only because it reaches its maximum at $\beta = 1$ and remains at this level across the entire fBm domain (Eke et al., 2000). The numerical descriptor of the fractal autocorrelation is the nearest-neighbor correlation coefficient, r_1 , which for fGn is given by $r_1 = 2^\beta - 1$ scaled between -0.5 to 0 (anticorrelation), and 0 (no correlation of random noise) to 1 (maximal correlation). The equivalent of correlation for fBm signals is persistence. From this point of view, one can differentiate between antipersistent (from $-fBm$ to $-fBm$) and persistent (from $+fBm$ to $++fBm$) signals, that are separated by the special case of random walk (at $\beta = 2$). These perplexity of terms can be simplified by referring all “correlated” signals ($+fGn$, $-fBm$, $-fBm$, $+fBm$, $++fBm$) as long-term memory processes (Achard et al., 2008; Wagenmakers et al., 2004), where the decay of autocorrelation with time is slower than the exponential decay.

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- We assessed fractality in BOLD signal fluctuations by the dichotomous $1/f^\beta$ model.
- Most signals were fGn, some fBm, the latter interferent with connectivity analysis.
- The difference between cortical and subcortical β s diminished *post mortem*.
- Near-zero *post mortem* β is due to deoxyhemoglobin impacting tissue water diffusion.
- Level of anesthesia decreased β in the cortex, only.

Figure 1. Internal structuring of spontaneous LFF of rat brain BOLD signal

(A) The raw BOLD signal has a (B) dominant frequency distribution between 0.001 Hz and 2.5 Hz. The double logarithmic representation of the spectrum shows 3 ranges: a very low frequency below 0.02 Hz, a mid-range between 0.02 and 0.3 Hz, where the power is decreasing in proportion to frequency (a scale-free, inverse power-law relationship, i.e. fractal feature) and a high frequency range, where the power is equally distributed across frequencies (random or white noise). By removing the very low and high frequency estimates from the spectrum (below and above the dotted lines on the left and right, respectively), (C) a time series and (D) its associated spectrum were obtained, capturing only scale-free distributed power ($|A|^2$) across frequencies (f) compatible with an $|A(f)|^2 \propto 1/f^\beta$ model, where β is the scaling exponent (see text for details). For the exemplary BOLD time course shown, the β value is 0.71.

Figure 2. Spectral characterization of fractality of the spontaneous LFF of rat brain BOLD signal

Time and frequency domain representations of single voxel fMRI data from two randomly chosen animals shown in upper and lower panels, respectively. (A) Data from one of the animals for in vivo and post mortem. (B) The same characteristics can be observed on the data series from another animal. Time course of the raw BOLD signal is 800 s long with resolution of 200 ms, whereas the frequency domain spectrum is plotted on a log-log (amplitude, $|A|^2$ vs. frequency, f) scale with a bandwidth of 0.001 to 1 Hz. The spontaneous LFF of the BOLD signal are modeled in the frequency domain as $|A(f)|^2 \propto 1/f^\beta$, where β is the scaling exponent (see text for details). Note that post mortem abolished the fractality in the signal.

Figure 3. Influence of data length and sampling rate in fractal analysis of rat brain BOLD signal
 (A) Effect of decreasing data length at constant sampling rate. (B) Effect of decreasing sampling rate at constant acquisition time. The color bars show the value of β , the scaling exponent in the frequency domain model of $|A(f)|^2 \propto 1/f^\beta$ (see text for details). The absolute lengths of corresponding image series were equal. Note that lower frequencies are better sampled by longer BOLD signals, whereas a high sampling rate is needed to capture dynamics in a wide bandwidth signal (Eke et al., 2002).

Figure 4. Stable topology of fractality in low-frequency resting-state BOLD signal fluctuations in the deeply anesthetized rat brain

Voxel-wise β values are plotted within a range of $0 < \beta < 1.4$. Please note, that "white noise" with $\beta=0$ and "1/f noise" with $\beta=1$ are only two special cases of the general model of spectral fractality, $|A(f)|^2 \propto 1/f^\beta$, the latter separating fractional Gaussian noise (fGn) and fractional Brownian motion (fBm) (see Appendix for details on fGn and fBm). (A) Repeated in vivo measurements of BOLD signal fluctuations from the onset of anesthesia shown with voxel-wise values of β (the scaling exponent of spectral fractality). Cortical and subcortical regions can be discerned by their respective β values, a pattern which is persistent through repeated measurements across a time span of 2 hours. (B) Overall topological stability of these β maps is represented by a mean β map which has little spatial variance at every voxel location as shown by the $\Delta\beta$ map. (C) Mean β maps for another two randomly chosen animals.

Figure 5. Physiological dependence of β values across different brain regions under deep anesthesia

(A) The anatomical MRI shows three selected regions of interest (ROIs) analyzed in the study: somatosensory forelimb area (S1FL), cingulate cortex (CG) and caudate putamen (CPu). BOLD signals were acquired under α -chloralose anesthesia. (B) Mean $\pm$ SD of β values in S1FL, CG, and CPu in vivo and post mortem. For each region, difference in β value between in vivo and post mortem were significant ($p < 0.01$), although β value in vivo for CPu was much smaller than that for S1FL and CG. Voxels with SNR < 30 dB were excluded from the analysis. Histograms of β values in S1FL, CG, and CPu (C) in vivo and (D) post mortem. In vivo the β distributions of S1FL and CG are partly overlapping with those of CPu, whereas post mortem all regions became greatly overlapping.

Figure 6. Physiological dependence of β values across different brain regions under light anesthesia

(A) Topology of in vivo measurements of β under domitor anesthesia is represented by a mean β map. (B) Mean $\pm$ SD of β values in S1FL, CG, and CPu in vivo and post mortem. For each region, difference in β value between in vivo and post mortem were significant ($p < 0.001$). Voxels with SNR < 30 dB were excluded from the analysis.