Research Article

NMR IN BIOMEDICINE

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Temporal scaling properties and spatial synchronization of spontaneous blood oxygenation level-dependent (BOLD) signal fluctuations in rat sensorimotor network at different levels of isoflurane anesthesia

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Spontaneous fluctuations in the blood oxygenation level-dependent (BOLD) MRI signal during the resting state are increasingly being studied in healthy and diseased brain in humans and animal models. Yet, the relationship between functional brain status and the characteristics of spontaneous BOLD fluctuations remains poorly understood. In order to obtain more insights into this relationship and, in particular, the effects of anesthesia thereupon, we investigated the spatial and temporal correlations of spontaneous BOLD fluctuations in somatosensory and motor regions of rat brain at different inhalation levels of the frequently applied anesthetic isoflurane. We found that the temporal scaling, characterized by the Hurst exponent (H), showed persistent behavior ($H > 0.5$) at 0.5–1.0% isoflurane. Furthermore, low-pass-filtered spontaneous BOLD oscillations were correlated significantly in bilateral somatosensory and bilateral motor cortices, reflective of interhemispheric functional connectivity. Under 2.9% isoflurane anesthesia, the temporal scaling characteristics approached those of Gaussian white noise ($H = 0.5$), the relative amplitude of BOLD low-frequency fluctuations declined, and cross-correlations of these oscillations between functionally connected regions decreased significantly. Loss of interhemispheric functional connectivity at 2.9% isoflurane anesthesia was stronger between bilateral motor regions than between bilateral somatosensory regions, which points to distinct effects of anesthesia on differentially organized neuronal networks. Although we cannot completely rule out a possible contribution from hemodynamic signals with a non-neuronal origin, our results emphasize that spatiotemporal characteristics of spontaneous BOLD fluctuations are related to the brain's specific functional status and network organization, and demonstrate that these are largely preserved under light to mild anesthesia with isoflurane.

Keywords: BOLD signal; resting state functional MRI; isoflurane anesthesia; rat brain; functional connectivity

INTRODUCTION

MRI measurements of spontaneous blood oxygenation level-dependent (BOLD) signal fluctuations allow the assessment of resting state brain activity, and hence may provide significant information on overall brain function (1–4). Synchronization of the low-frequency part of spontaneous BOLD oscillations is believed to reflect functional connectivity, a measure of spatiotemporal correlation between distinct brain regions (1–4). However, the relationship between functional brain status and the characteristics of spontaneous BOLD fluctuations remains incompletely understood.

To elucidate the physiological origin and functional significance of resting state functional MRI (fMRI) signals, several studies have assessed BOLD fluctuations under altered states of neural activity. For example, coherent spontaneous BOLD signal oscillations have been found to be preserved in various states of unconsciousness, e.g. during light sleep (5,6) or under anesthesia (7). However, loss of interhemispheric resting state functional connectivity has been observed in healthy sevoflurane-anesthetized subjects (8) and in α-chloralose-anesthetized rats (9). Loss of interhemispheric

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Abbreviations used: *ANOVA, analysis of variance; BOLD, blood oxygenation level-dependent; CBF, cerebral blood flow; CPu, caudate putamen; EEG, electroencephalography; fMRI, functional MRI; H, Hurst exponent; LSD, least-significant difference; M1, primary motor cortex; M2, secondary motor cortex; MAC, minimum alveolar concentration; ROI, region of interest; S1FL, forelimb region of the primary somatosensory cortex; S2, secondary somatosensory cortex; SD_{LFBF}, standard deviation of low-frequency BOLD fluctuations; VPL, ventral posterolateral thalamic nucleus.*

connectivity has also been found in a resting state fMRI study on a minimally conscious patient (10). This suggests that, although there may be coherent network activity reflective of basic functional brain organization, the spatial correlation pattern of spontaneous fMRI signal oscillations is related to the level of neural activity. This may be particularly relevant for resting state fMRI studies that are conducted under anesthesia, such as in patients and animal models.

Our aim was to characterize the effect of different levels of a frequently applied volatile anesthetic, isoflurane, on the temporal properties of spontaneous BOLD signal alterations and the spatial correlation of BOLD low-frequency fluctuation signals in sensorimotor brain regions in rats. Concordant with a loss of coherence of neuronal signals in the brain, we hypothesized that increased isoflurane anesthesia levels would reduce the fractal properties of BOLD signal time series, characterized by the Hurst exponent (H) [11,12], as well as the functional connectivity within sensorimotor networks.

EXPERIMENTAL DETAILS

Animals

All animal procedures were approved by the local ethical committee of Utrecht University and met governmental guidelines. Eight male Wistar rats, weighing 250–280 g, were included in the study. Prior to MRI, rats were anesthetized with 4% isoflurane for endotracheal intubation, followed by mechanical ventilation with 1.8% isoflurane in air–O_2 (2 : 1). We monitored end-tidal CO_2 and adjusted the ventilation frequency between 52 and 59 beats per minute to prevent hypo-/hypercapnia.

MRI data acquisition

MRI measurements were performed on a 4.7-T, 40-cm horizontal bore MR system (Varian, Palo Alto, CA, USA) with a gradient-coil insert (internal diameter, 125 mm; maximum gradient strength, 500 mT/m; rise time, 110 μs) (Magnex Scientific, Oxford, UK). A Helmholtz volume coil (diameter, 90 mm) and an inductively coupled surface coil (diameter, 25 mm) were used for signal excitation and detection, respectively. Rats were placed in an MR-compatible stereotaxic holder and immobilized with earplugs and a toothholder.

Multi-echo, T_2-weighted MRI (TR = 3000 ms; TE = 17.5 ms; echo train length, 8; field of view, $32 \times 32\ mm^2$; acquisition matrix, 128×128; 19 1-mm slices) was conducted for anatomical information. BOLD MRI was conducted with a gradient-echo echo planar imaging sequence (flip angle, 50°; TR = 1000 ms; TE = 19 ms; field of view, $32 \times 32\ mm^2$; acquisition matrix, 64×64, zero-filled to 128×128; 13 1-mm slices; 600 acquisitions; total acquisition time, 10 min). Three successive resting state fMRI measurements were performed with end-tidal isoflurane at 1%, 2.9% and 0.5%, respectively. Time intervals of 15 min were incorporated to allow for transition to the next isoflurane level.

During MRI, blood oxygen saturation and heart rate were monitored. Body temperature was maintained at 37.0 ± 0.5°C.

Data preprocessing

For optimal stability of the resting state fMRI time series signal, we removed the first 30 images. To correct for interscan motion, the remaining 570 images were realigned to the first volume using statistical parametric mapping (SPM2, http://www.fil.ion.ucl.ac.uk/spm/). To reduce potential non-neuronal contributions and to improve the specificity of functional connectivity assessment (13), linear drift was de-trended and other spurious variations were removed using multiple regression with the head motion parameters and whole-brain mean signal as regressors. The removal of the global brain signal has been put forward as an effective data preprocessing step to suppress cardiac- and respiratory-related BOLD signal variance (13). The functional images were coregistered with the T_2-weighted anatomical images. On coregistration of the stereotaxic rat brain atlas of Paxinos and Watson (14) to the anatomical image of each rat, six bilateral regions of interest (ROIs) were selected: primary motor cortex (M1), secondary motor cortex (M2), forelimb region of the primary somatosensory cortex (S1FL), secondary somatosensory cortex (S2), ventral posterolateral thalamic nucleus (VPL) and caudate putamen (CPu) (see Fig. 1).

Data analysis: temporal characteristics of BOLD signals

Biological systems, such as the brain, have fractal properties in space and time (15–17). Fractal signals are typically long-memory processes with a slowly decaying autocorrelation function (18). In the frequency domain, this corresponds to a 1/*f*-like spectral density function, with the lower frequencies having greater power; the slope of the straight line fitted to the logarithm of the periodogram is defined as the spectral exponent γ, i.e. $S(f) = f^{-\gamma}$ or $\log S(f) = -\gamma \log f$. The spectral exponent γ is related to the fractal dimension D [$D = (3 - \gamma)/2$] and the Hurst exponent H [$H = (\gamma + 1)/2$] of the process (18). $H = 0.5$ corresponds to classical Gaussian white noise. If $0 < H < 0.5$, the autocovariance is negative and the signal is anticorrelated, whereas, if $0.5 < H < 1$, the autocovariance is positive and the signal has long memory or positive autocorrelation over long time lags.

There are alternative estimators of H in the wavelet domain with low bias and high efficiency (11,12). The band-pass property of wavelet filters implies that, for a discrete signal with a 1/*f*-like power spectrum, the wavelet coefficients at each scale are decorrelated and coefficients at different scales are uncorrelated for any wavelet basis (19). Therefore, at each scale $j = 1, 2, \ldots J$, the wavelet coefficients of the discrete wavelet transform can be regarded as independent identically distributed Gaussian

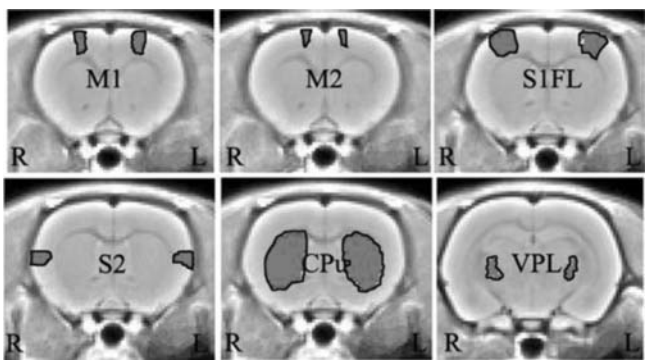

Figure 1. Regions of interest (ROIs) depicted on coronal slices from a stereotaxic average rat brain MRI template. M1, primary motor cortex; M2, secondary motor cortex; S1FL, forelimb region of the primary somatosensory cortex; S2, secondary somatosensory cortex; CPu, caudate putamen; VPL, ventral posterolateral thalamic nucleus.

variables with zero mean and variance σ_j^2. The quantity $\log(\sigma_j^2)$ is a linear function of j, and an estimate of H may be obtained by regression of the log-variance of the wavelet coefficients with respect to scale (12).

Using the wavelet maximum likelihood estimator (12), we calculated the H value of the average BOLD time series for each ROI during ventilation with different isoflurane concentrations to see whether the temporal scaling properties of the spontaneous BOLD signal were dependent on the level of anesthesia.

Data analysis: spatial characteristics of low-frequency BOLD signals

To investigate the spatial correlations of spontaneous low-frequency BOLD fluctuations, the fMRI waveform of each voxel was temporally band-pass filtered ($0.01\,\text{Hz} < f < 0.08\,\text{Hz}$). To quantify the functional connectivity, we first calculated the Pearson's correlation coefficients between two low-pass-filtered BOLD signal time series, and then transformed the r value to the Z value using Fisher's r to Z transformation. For each ROI, we analyzed two kinds of functional connectivity: (i) within the ROI: the mean of the Z values between the low-frequency BOLD fluctuations of each voxel within the ROI and the average low-frequency BOLD signal time series of the ROI; (ii) between bilateral ROIs: the Z value between the average low-frequency BOLD oscillations of the left and right ROIs. For each ROI, we also measured the mean relative amplitude of the low-frequency BOLD fluctuations by calculating the standard deviation of the low-pass-filtered BOLD signal time series (SD_{LFBF}).

To assess the correlations between the spatial and temporal characteristics of the BOLD signals, we calculated the linear correlation coefficient between the functional connectivity (Z value of cross-correlation between the average low-frequency BOLD time series signals of the left and right ROIs) and the representative H value of each pair of bilateral ROIs [measured by averaging the Hurst exponents of the two reference signals (average time series of the left and right ROIs, respectively)]. For adequate statistical power, all data points acquired at different isoflurane levels were included.

Statistics

All data are presented as the mean $\pm$ SD. One-way repeated-measures analysis of variance (ANOVA) and *post hoc* Tukey's least-significant difference (LSD) test were used to analyze differences in H, Z and SD_{LFBF} values between different anesthesia levels. $p < 0.05$ was considered to be significant.

RESULTS

Successful transition to different levels of anesthesia was confirmed by significant changes in heart rate (410 ± 33, 402 ± 33 and 371 ± 31 beats per minute at 0.5%, 1.0% and 2.9% isoflurane, respectively).

Temporal characteristics of BOLD signals

Figure 2 shows the BOLD time series and corresponding Hurst exponent (H) values from a typical rat at different isoflurane anesthesia levels. For all voxels outside the brain, H values were 0.50 ± 0.05, 0.51 ± 0.06 and 0.51 ± 0.05 under 0.5%, 1.0% and 2.9% isoflurane, respectively, suggesting that the background noise was white. The mean signal of the left and right S1FL demonstrated $H > 0.5$ at all three anesthesia levels. However, with increasing anesthesia levels, H decreased significantly and approached the level of Gaussian white noise, which is shown in Fig. 3 for all left and right ROIs. At 2.9% isoflurane, H values approximated 0.5 and were significantly lower than at 0.5% isoflurane in cortical ROIs and the CPu.

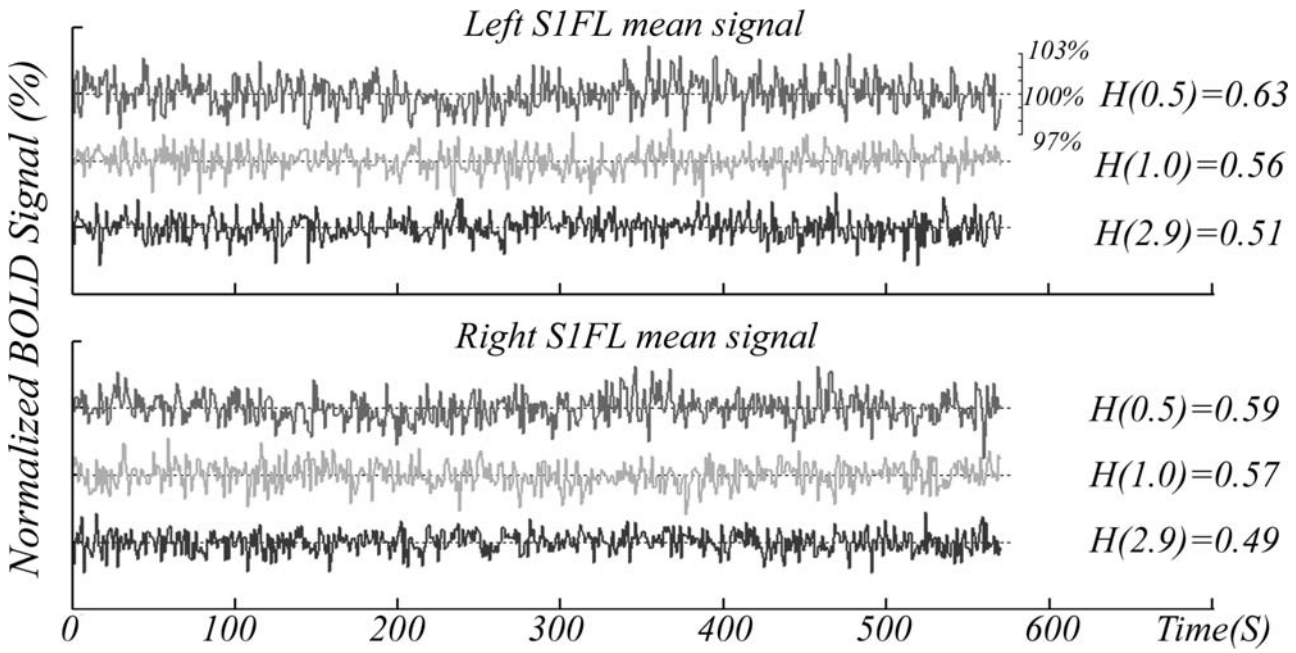

Figure 2. Mean blood oxygenation level-dependent (BOLD) signal time series and corresponding Hurst exponents (H) in left (top) and right (bottom) forelimb region of the primary somatosensory cortex (S1FL) at three isoflurane anesthesia levels (0.5%, dark gray lines; 1.0%, light gray lines; 2.9%, black lines) from a randomly selected rat.

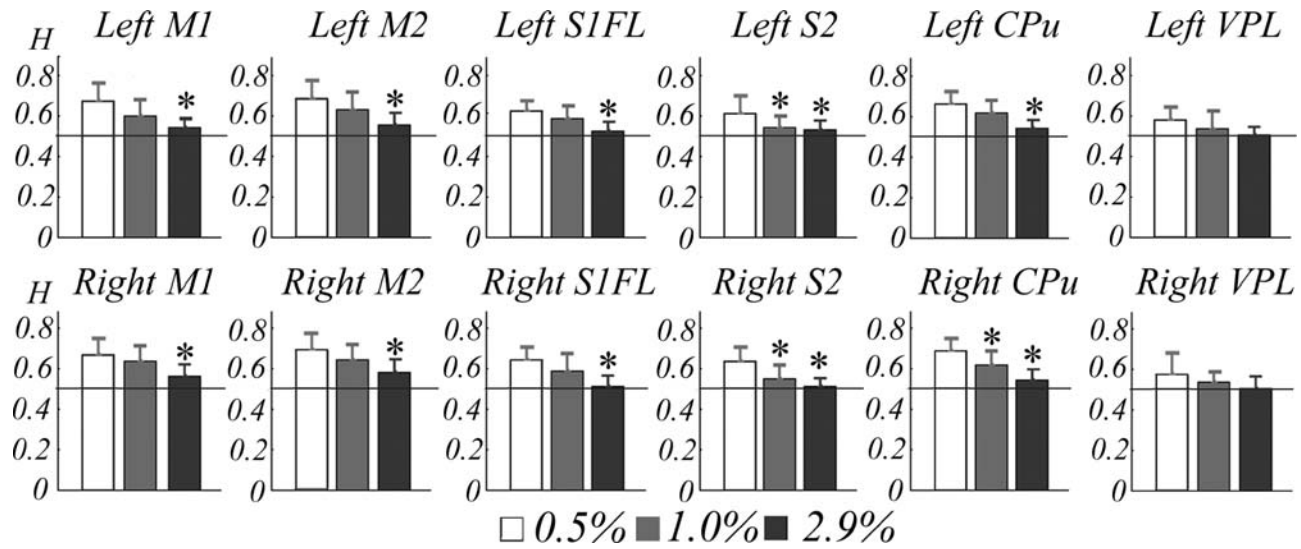

Figure 3. Hurst exponents (*H*) (mean ± SD, $n = 8$) calculated from blood oxygenation level-dependent (BOLD) signal time series for each region of interest (ROI) at 0.5% (white bars), 1.0% (gray bars) and 2.9% (black bars) isoflurane anesthesia. $^{*}p < 0.05$ *vs* 0.5%. M1, primary motor cortex; M2, secondary motor cortex; S1FL, forelimb region of the primary somatosensory cortex; S2, secondary somatosensory cortex; CPu, caudate putamen; VPL, ventral posterolateral thalamic nucleus.

Spatial characteristics of low-frequency BOLD signals

Low-frequency BOLD oscillations in left and right cortical ROIs were correlated significantly at 0.5% and 1.0% isoflurane anesthesia, as shown for M1 in Fig. 4. The displayed functional connectivity maps were calculated from the cross-correlation between the average BOLD time series of one of the unilateral M1 ROIs and all voxels within the brain. The maps clearly demonstrate functional connectivity between seed region M1 and other sensorimotor cortical regions (M1, M2, S1, S2) in both hemispheres at low anesthesia levels. At the higher anesthesia level, interhemispheric connectivity was clearly reduced. Indeed, the number of voxels having a significant correlation with the reference time course decreased in both hemispheres.

Figure 5 shows the group results from functional connectivity analyses as calculated from cross-correlation coefficients for low-frequency BOLD signals. Functional connectivity within all ROIs was reduced significantly at the highest anesthesia percentage (except for VPL where the BOLD signal-to-noise ratio was relatively low). Functional connectivity between bilateral ROIs also decreased towards higher isoflurane levels. A significant loss of interhemispheric functional connectivity under 2.9% isoflurane, relative to 0.5% isoflurane, was measured for bilateral M1, M2 and CPu. At the group level, we also found that SD_{LFBF} in the ROIs declined significantly with increasing anesthesia levels ($p < 0.05$).

Cross-correlation coefficients between bilateral ROIs were correlated significantly with the averaged Hurst exponent of the two ROIs ($p < 0.05$). The linear correlation coefficients were 0.65, 0.57, 0.50, 0.75, 0.52 and 0.45 for M1, M2, S1FL, S2, CPu and VPL, respectively.

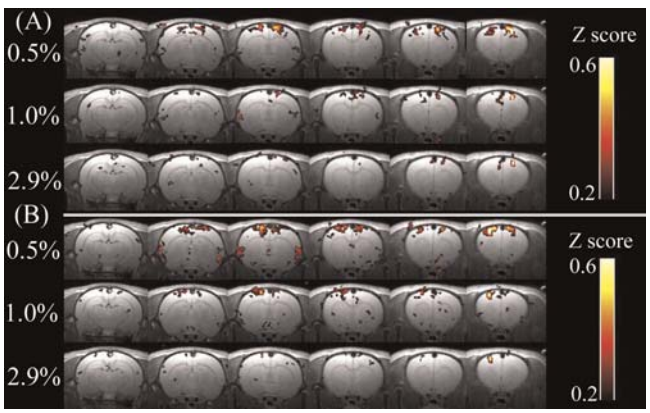

Figure 4. Maps of functional connectivity with left (A) or right (B) primary motor cortex (M1) in a randomly selected rat anesthetized with different isoflurane percentages. Rows display T_2-weighted MR images of consecutive coronal brain slices from posterior to anterior (left to right). Color coding denotes voxels with significant correlation with the reference low-frequency blood oxygenation level-dependent (BOLD) fluctuations in left or right M1 ($Z > 0.2$, $p < 10^{-5}$).

DISCUSSION

The analysis of spontaneous activity during BOLD MRI is becoming a popular method to investigate brain function in health and disease. However, the effect of altered functional status of the brain, such as under anesthesia, on spontaneous BOLD signal fluctuations is still largely unclear. Our study attempted to characterize the spatial and temporal correlations of spontaneous BOLD signal fluctuations in rat brain under different levels of isoflurane ventilation to gain an insight into the relationship between anesthesia and resting state fMRI signals in the brain.

First, the temporal characteristics of the BOLD signal time series were evaluated by calculation of the Hurst exponent (*H*), which may reflect hemodynamic features associated with long-term trends in neuronal activity. Recent resting state fMRI studies have described long memory processes in the human brain (12,20), and *H* values of time series from human fMRI data have been shown to correlate positively with the degree of neural activity (21). In our study on anesthetized rat brain, *H* was close to 0.5 for all voxels outside the brain, whereas we observed

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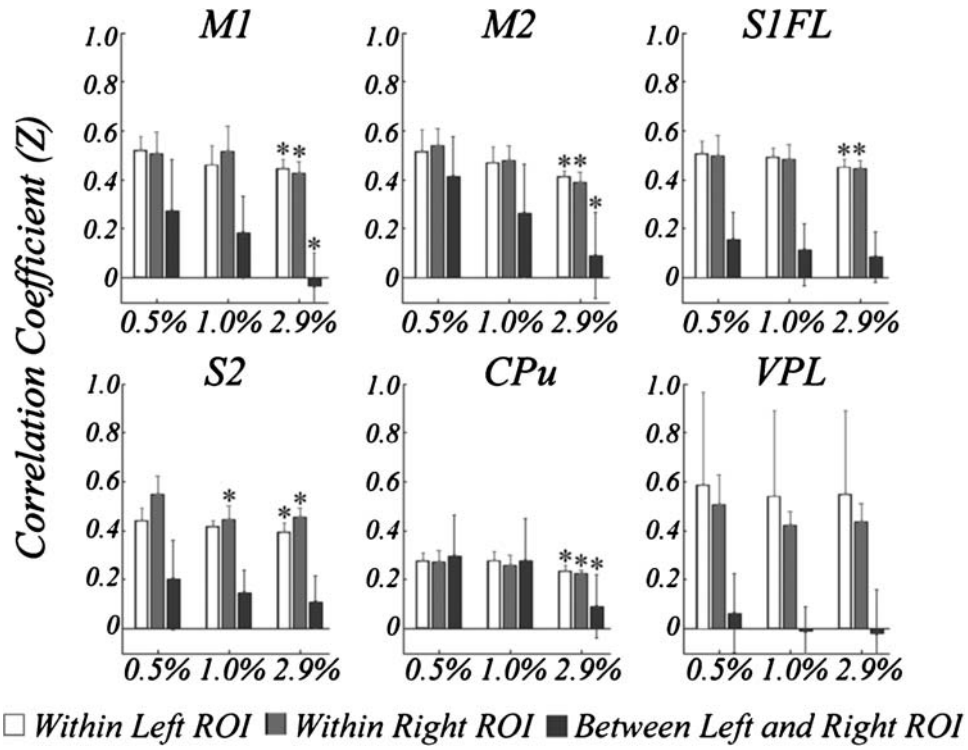

Figure 5. Functional connectivity [*Z* values of cross-correlations between low-frequency blood oxygenation level-dependent (BOLD) fluctuations; mean ± SD, $n = 8$] within left (white bars) and right (gray bars) regions of interest (ROIs), and between bilateral ROIs (black bars), at three different isoflurane anesthesia levels (%). $^{*}p < 0.05$ *vs* 0.5%. M1, primary motor cortex; M2, secondary motor cortex; S1FL, forelimb region of the primary somatosensory cortex; S2, secondary somatosensory cortex; CPu, caudate putamen; VPL, ventral posterolateral thalamic nucleus.

persistent behavior of spontaneous BOLD fluctuations ($H > 0.5$) in brain ROIs at 0.5% and 1.0% isoflurane anesthesia. It is important to realize that various nonphysiological and non-neuronal physiological signals can also cause BOLD fluctuations. Nevertheless, we found that *H* values in voxels outside the brain were around 0.5 (Gaussian white noise) and stable across different anesthesia levels. This suggests that machine noise and other environmental disturbances were not the origin of the persistent scaling behavior in the brain signal, and that there was still meaningful spontaneous neurophysiological activity at isoflurane levels corresponding to light anesthesia. This is consistent with electrophysiology experiments, which have demonstrated that activation, as measured by electroencephalography (EEG), can be elicited in rats at isoflurane levels below one minimum alveolar concentration (MAC) [for rats 1.0 MAC corresponds to 1.4–1.5 vol.% (22)] (23). When isoflurane was increased to 2.9%, *H* was approximately 0.5 in all brain ROIs, which suggests that the underlying mechanisms of spontaneous activity are disrupted under deeper anesthesia, causing spontaneous BOLD signals to be indistinguishable from background noise. This is in agreement with the finding of EEG suppression to isoelectricity with periodic burst activity at isoflurane levels above 1 MAC in rats (24). Nonetheless, it should be noted that direct vasomotor effects of isoflurane could also influence the pattern of spontaneous BOLD oscillations. As a vasodilator, isoflurane increases cerebral blood volume and cerebral blood flow (CBF), which may affect the relationship between hemodynamics and neuronal activity. Yet, coupling of cerebral glucose utilization and CBF has been shown to be maintained between 0 and 1.0 MAC isoflurane, albeit shifted towards higher CBF levels (25,26). At 2.0 MAC (*c.* 2.9 vol.%), the relationship between CBF and metabolism is reduced in some brain areas, but largely present in the majority of the brain, including the cortex (26). Furthermore, a significant correlation has been found between CBF oscillations and the pattern of electrocortical activity in isoflurane-anesthetized rats, which persisted when isoflurane levels were increased above 1 MAC, despite a clear drop in the frequency of electrical bursts and spontaneous CBF waves (27). Nevertheless, we cannot rule out the possibility that isoflurane-induced modification of vascular oscillations with a non-neuronal, myogenic origin may contribute to the detected changes in *H* in the brain. For example, the observed decrease in amplitude of the low-frequency BOLD fluctuations with increasing isoflurane levels may be partly caused by suppression of vasomotor dynamics.

Second, our data on the spatiotemporal synchronization of spontaneous BOLD oscillations indicate that resting state functional connectivity of bilateral homologous regions in rats is preserved under 0.5% and 1.0% isoflurane anesthesia. This is in accordance with previous studies in <1.0 MAC isoflurane-anesthetized monkeys (7) and rats (28), as well as medetomidine-sedated rats (29,30), indicating that the spatial coherence of the low-frequency part of the spontaneous BOLD fluctuations is largely maintained in lightly to mildly anesthetized animal brain. Furthermore, we detected a decline of functional connectivity between bilateral sensorimotor ROIs with higher doses of isoflurane anesthesia, which agrees with the loss of interhemispheric EEG synchronization as measured in rat frontal cortex with increasing isoflurane levels (31). Similar resting state fMRI findings have been reported for sevoflurane-anesthetized human subjects (8) and α-chloralose-anesthetized rats (9). Lu *et al.* (9)

found that the cross-correlation between low-frequency BOLD fluctuations in the left and right forelimb regions of the somatosensory cortex decreased at higher infusion doses of α-chloralose. Interestingly, we found that the loss of interhemispheric functional connectivity at 2.9% isoflurane anesthesia was stronger between bilateral motor regions than between bilateral somatosensory regions. The anesthetic effect of isoflurane has been linked to the disruption of functional interaction within the corticothalamocortical circuit (32). As descending corticothalamic connections outnumber their ascending thalamocortical counterparts (33), we speculate that functional disconnection between the thalamus and cortex may have a larger impact on the coherence of signals in motor cortical regions. In addition, the loss of signal synchronization between bilateral cortical regions may be caused by disruption of structural interhemispheric connection through the corpus callosum. A significant decline in glucose utilization at 2.0 MAC isoflurane has been detected in rat corpus callosum (25). In rats, callosal connectivity is higher between bilateral M1 regions than between bilateral S1 regions (34,35). Thus, metabolic depression of interhemispheric corticocortical connectivity at high isoflurane levels may also have accounted for the observed larger loss of functional connectivity between bilateral motor regions.

In addition to the above-described anesthesia-induced reduction in functional connectivity between homologous ROIs, we also found that the degree of local functional connectivity within ROIs was inversely related to the anesthesia level. Although our finding of an anesthesia dose-dependent decline in interhemispheric connectivity agrees with the article by Lu *et al.* (9), these authors observed maintained intrahemispheric synchronization across α-chloralose doses. This may be explained by distinctive effects of the different anesthetic agents. Isoflurane anesthesia above 1 MAC depresses the cerebral metabolic rate (25,26) and EEG activity in sensorimotor cortex (24), and our data suggest that this is accompanied by reduced coherence of low-frequency BOLD oscillations. The loss of intra- and interhemispheric functional connectivity concurs with the above-described *H* values being close to 0.5, and the degree of functional connectivity between bilateral ROIs was shown to correlate with the average *H* value of the two ROIs. This implies that spontaneous BOLD fluctuations under moderate to deep anesthesia approximate meaningless noise, resulting in spatial decoherence in the brain.

CONCLUSION

In this work, we evaluated the alterations in temporal and spatial properties of spontaneous BOLD signal fluctuations under different isoflurane anesthesia levels in rats. We found that the persistent behavior and spatial coherence of spontaneous BOLD oscillations were maintained at 0.5% and 1.0% isoflurane. Together with an increase in the anesthetic level, the temporal scaling properties approached that of trivial white noise, and the spatial coherence also decreased. This suggests that resting state fMRI characteristics that are reflective of intrinsic brain organization are largely preserved at light to mild levels of anesthesia with isoflurane, but disappear under deeper anesthesia.

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