

Global reduction of information exchange during anesthetic-induced unconsciousness

Christina Hamilton¹ · Yuncong Ma² · Nanyin Zhang^{1,2} 

Received: 28 July 2016 / Accepted: 26 February 2017 / Published online: 13 March 2017
© Springer-Verlag Berlin Heidelberg 2017

Abstract During anesthetic-induced unconsciousness (AIU), the brain undergoes a dramatic change in its capacity to exchange information between regions. However, the spatial distribution of information exchange loss/gain across the entire brain remains elusive. In the present study, we acquired and analyzed resting-state functional magnetic resonance imaging (rsfMRI) data in rats during wakefulness and graded levels of consciousness induced by incrementally increasing the concentration of isoflurane. We found that, regardless of spatial scale, the functional connectivity (FC) change (i.e., ΔFC) was proportionally dependent on the FC strength at the awake state across all connections. This dependency became stronger at higher doses of isoflurane. In addition, the *relative* FC change at each anesthetized condition (i.e., ΔFC normalized to the corresponding FC strength at the awake state) was exclusively negative across the whole brain, indicating a global loss of meaningful information exchange between brain regions during AIU. To further support this notion, we showed that during unconsciousness, the entropy of rsfMRI signal increased to a value comparable to random noise while the mutual information decreased appreciably. Importantly, consistent results were obtained when unconsciousness was induced by dexmedetomidine, an anesthetic

agent with a distinct molecular action than isoflurane. This result indicates that the observed global reduction in information exchange may be agent invariant. Taken together, these findings provide compelling neuroimaging evidence suggesting that the brain undergoes a widespread disruption in the exchange of meaningful information during AIU and that this phenomenon may represent a common system-level neural mechanism of AIU.

Keywords Anesthetic-induced unconsciousness · Resting-state functional connectivity · Isoflurane · Dexmedetomidine · Rat

Introduction

For over a century, the neural mechanisms of consciousness have been explored in the neuroscience field (Alkire et al. 2008). One common strategy to investigate such a phenomenon is the study of altered forms of consciousness as modulated in pharmacological, physiological, or pathological conditions. In particular, anesthetic-induced unconsciousness (AIU) is an intriguing avenue for investigating consciousness given the convenience of experimental control and the essential role of anesthesia in modern medicine. AIU is a temporary and reversible loss of consciousness (LOC) produced by the administration of anesthetic agents and accompanied by analgesia, amnesia, and immobility (Brown et al. 2010). Although the molecular actions of various anesthetic agents are fairly well understood, the system-level neural mechanisms of AIU remain obscure.

Accumulating evidence suggests that AIU is a brain network phenomenon. Anesthetics appear to suppress consciousness by disrupting information exchange across large-scale brain networks (Alkire et al. 2000;

Christina Hamilton and Yuncong Ma contributed equally to this work.

✉ Nanyin Zhang
nuz2@psu.edu

¹ The Huck Institutes of Life Sciences, The Pennsylvania State University, University Park, PA 16802, USA

² Department of Biomedical Engineering, The Pennsylvania State University, W-341 Millennium Science Complex, University Park, PA 16802, USA

Tononi 2004; Mashour and Alkire 2013; Mashour 2014; Lee et al. 2013; Liang et al. 2012b; Boveroux et al. 2010; Martuzzi et al. 2010). This notion has been supported by a number of studies in both humans (White and Alkire 2003; Boveroux et al. 2010; Deshpande et al. 2010; Martuzzi et al. 2010) and animals (Vincent et al. 2007; Moeller et al. 2009; Wang et al. 2011; Liang et al. 2013), which have indicated that a disruption of functional connectivity (FC) specifically within the thalamocortical and frontoparietal networks is essential to AIU (Angel 1991; Velly et al. 2007; Lee et al. 2009; Breshears et al. 2010; Imas et al. 2005).

While these previous studies have highlighted the importance of thalamocortical and frontoparietal networks in AIU, whether and how the rest of the brain is involved during AIU remains unclear. The answer to this question will provide critical insight for understanding whether the effects anesthetics are limited in specific neural networks (e.g., the thalamocortical network), or whether AIU leads to information exchange loss/gain across the whole brain. To address this issue, it is essential to elucidate the FC changes across whole-brain networks during AIU. The importance of such investigation is also highlighted by the inter-connected characteristics of the brain organization, in which *global* coordination is critical for effective information exchange (Moon et al. 2015; Liang et al. 2012b). In addition, most anesthetic agents affect neurotransmitters throughout the entire brain (Franks 2008; Brown et al. 2010). Therefore, unraveling the effects of anesthesia on the whole-brain network can be critical for deciphering the system-level neural mechanisms of AIU.

In the present study, we employed the technique of resting-state functional magnetic resonance imaging (rsfMRI) to investigate information exchange loss/gain across the whole rat brain during wakefulness and graded levels of consciousness induced by increasing concentrations of isoflurane. rsfMRI uses the temporal correlation of low-frequency spontaneous fluctuations of the blood oxygenation-level-dependent (BOLD) signal as a measure of FC between brain regions (Biswal et al. 1995). Our data revealed that relative to the awake state, the normalized FC changes during isoflurane-induced unconsciousness were exclusively negative across the whole brain, which indicates that the disruption of information transfer is ubiquitous in the brain. In addition, this pattern of information exchange loss was similar during AIU induced by a distinct anesthetic agent, dexmedetomidine. Collectively, these data suggest that the global disruption of information exchange may be an agent-invariant mechanism of AIU.

Methods

Animal preparation

Thirty-seven adult male Long-Evans rats (300–500 g) were housed and maintained on a 12-h light: 12-h dark schedule, and provided access to food and water ad libitum. Approval for the study was obtained from the Institutional Animal Care and Use Committee of the Pennsylvania State University. Animals were first acclimated to the scanning environment for 7 days to minimize stress and motion during imaging at the awake state [described in Liang et al. (2011, 2012a, b, 2013, 2014, 2015a, b), and Zhang et al. (2010)]. To do this, rats were restrained and placed in a mock MRI scanner where prerecorded MRI sounds were played. The exposure time in the mock scanner started from 15 min on day 1 and was incrementally increased by 15 min each day up to 60 min (days 4, 5, 6, and 7). This setup mimicked the scanning condition inside the magnet.

Behavioral assessment

The animal's consciousness level was assessed using the behavioral test of loss of right reflex (LORR), which is a well-established index of unconsciousness in rodents. A strong correlation has been established between the concentration of various anesthetic agents needed to produce the loss of voluntary movement in rodents and loss of consciousness in humans (Franks 2008). In the LORR procedure, each rat began in a restrained, awake state (i.e., 0% isoflurane) and was exposed to increasing doses of isoflurane via a nosecone. We measured the LORR for a single dose delivered to the rat exactly as it would be in the scanner (Fig. 1): each scan was 10 min with a 5-min transition time between scans to allow the isoflurane concentration to reach a steady state. Thus, for example, to measure LORR

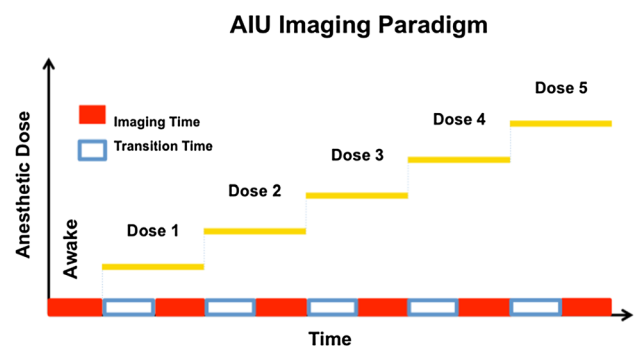


Fig. 1 A schematic of the AIU imaging paradigm. Imaging began while animals were in a fully awake state, followed by five doses of increasing concentrations of isoflurane. For each dose, a 5-min transition period was given before the rsfMRI scan started to allow the isoflurane concentration to reach steady state

at 1.5% isoflurane, the animal first received 0.5% isoflurane for 15 min, then 1.0% isoflurane for 15 min, and finally 1.5% isoflurane for 5 min. With the nosecone in place, the rat was then taken out of the restrainer and turned over to a supine position, while the time it took to correct its position was recorded. If the rat did not correct its position within 60 s, it was deemed completely unconscious. By mimicking anesthesia administration during the scanning process, this procedure allowed us to measure the animal's consciousness level at each anesthetic dose administered during rsfMRI scanning.

MRI experiments

Rats were briefly anesthetized with isoflurane while they were placed in a head restrainer with a built-in birdcage volume coil. Isoflurane was discontinued, but the nosecone remained fastened around the animal's nose for the duration of the experiment. Imaging began 30 min after rats were placed in the scanner, while animals were fully awake. Image acquisition was performed on a 7 T scanner. A high-resolution T1 structural image was first acquired with the following parameters: repetition time (TR)=2125 ms, echo time (TE)=50 ms, matrix size=256×256, field of view (FOV)=3.2 cm×3.2 cm, slice number=20, and slice thickness=1 mm. rsfMRI data were then obtained during the awake state and graded levels of consciousness by systematically increasing the concentration of isoflurane from 0% (i.e., awake state) to 3% (0, 0.5, 1.0, 1.5, 2.0, and 3.0%). Isoflurane was administered via the nosecone. rsfMRI data were acquired using a single-shot gradient-echo, echo planar imaging pulse sequence with TR=1000 ms, TE=13.8 ms, flip angle=80°, matrix size=64×64, FOV=3.2×3.2 cm, slice number=20, and 1-mm-thick slices (in-plane resolution=500 μm×500 μm). For each rsfMRI scan, 600 volumes were acquired in 10 min. Between each dose, 5 min were allowed to ensure the new dosage reached steady state (see Fig. 1).

Image preprocessing

The first ten volumes of each rsfMRI run were discarded to allow magnetization to reach steady state. The data were preprocessed with conventional procedures as previously described (Liang et al. 2014, 2015a, b), which include: registration to a segmented rat brain atlas, motion correction with SPM12, spatial smoothing (FWHM=1 mm), regression of nuisance signals, as well as band-pass filtering (0.01–0.1 Hz). 16 nuisance signals were regressed out, including the signals from white matter (WM), cerebrospinal fluid (CSF), 6 motion parameters, and their derivatives. This regression strategy has been reported to minimize the impact of motion artifacts

on rsfMRI data (Power et al. 2015). We chose not to regress out the global signal to avoid any spurious anti-correlations that may arise (Murphy et al. 2009).

Data analysis

Data were separately analyzed on a region-of-interest (ROI) or voxel basis. For the ROI-based analysis, we first parcellated the brain into 134 unilateral ROIs based on the anatomic definition of brain regions in Swanson Atlas (Swanson 2004). We derived FC by correlating the BOLD time series of each pair of ROIs. To ensure that our results were independent of the parcellation scheme and spatial scale, we also conducted the voxel-based analysis by calculating the FC between each pair of voxels within the brain. For each connection (between a pair of ROIs or between a pair of brain voxels), its FC change (ΔFC) was determined by subtracting the FC strength at the awake state from the corresponding FC strength at each anesthetized condition. In addition, ΔFC at each dose was divided by the corresponding FC strength at the awake state (referred to as normalized ΔFC hereafter). Furthermore, to examine the change of information exchange for a local brain region (a voxel or ROI), we averaged the normalized ΔFC between this voxel (or ROI) and all other brain voxels (or ROIs) at each anesthetized condition (Olde Dubbelink et al. 2013). This averaged normalized ΔFC was used as the quantity to measure information exchange loss/gain for the voxel/ROI.

To test our hypothesis that spontaneous brain activity becomes more random during AIU as a result of the loss of meaningful information processing, we calculated the Shannon entropy of the BOLD time series for each ROI as well as the mutual information between each pair of ROIs at each condition. Entropy represents the expected uncertainty (or randomness) of the information source, and mutual information is the reduction of this uncertainty. In the present study, entropy characterizes the information contained in the BOLD signal, while mutual information represents the information exchanged between brain regions. To estimate the entropy distribution of random noise for the purpose of comparison, we simulated noise using a normal distribution and smoothed the noise using the same temporal filter used in data preprocessing. All data analyses were conducted using Mathematica software (Wolfram, Champaign, IL, USA).

All imaging and behavioral data (LORR) were also used in a separate study (Ma et al. 2016), which examined the dynamic connectivity patterns in conscious and unconscious brain. All data were reanalyzed for a different purpose specifically for this study.

Results

In the current study, we examined the anesthetic-induced changes in information exchange across the whole brain as animals transitioned from an awake state to an unconscious state. rsfMRI data during wakefulness and at graded levels of consciousness produced by increasing concentrations of isoflurane (0.5, 1.0, 1.5, 2.0, and 3.0%) were collected in rats.

Animals lost consciousness at 1.5% isoflurane

To assess the animal's consciousness level at each dose, we first measured the LORR outside of the scanner in a manner that directly mimicked the way the animal experienced the dose during rsfMRI scanning (Fig. 1, also see "Methods" section for details). We found that when 1.5% isoflurane was administered via a nosecone, 81% of animals were unable to correct from the supine to prone position.

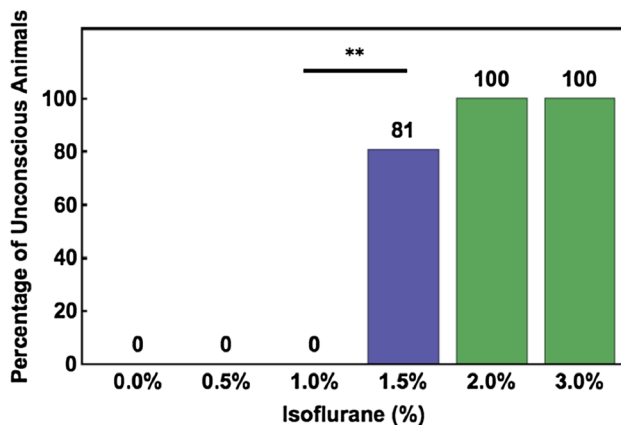


Fig. 2 LORR test in isoflurane-anesthetized animals. The percentage of animals that were unable to correct from a supine to a prone position was measured at multiple concentrations of isoflurane: 0% (i.e., the awake state), 0.5, 1.0, 1.5, 2.0, and 3.0%. This percentage significantly increased from 1.0 to 1.5% (Chi square test, $\chi^2=22.82$, $p<2\times 10^{-6}$), indicating that most animals lost consciousness at the isoflurane concentration of 1.5% (delivered through a nosecone)

This percentage was significantly higher than when lower doses of isoflurane were administered (no rats lost righting reflex at lower doses, Fig. 2, Chi square test, $\chi^2=22.82$, $p<2\times 10^{-6}$). Therefore, 1.5% was the dose to induce LOC when isoflurane was delivered through a nosecone. Notably, this dose was higher than isoflurane doses inducing LORR reported in other studies (0.7–0.8%), in which animals were either intubated or were placed in an enclosed chamber (MacIver and Bland 2014; Hudetz et al. 2011). This apparent contradiction was due to the different setup used in the present study (i.e., isoflurane was delivered via a nosecone), in which the actual concentration of isoflurane in the animal was lower than the concentration delivered due to air dilution around the nose cone.

FC was significantly disrupted in the thalamocortical and frontoparietal networks

Numerous literature studies (White and Alkire 2003; Boveroux et al. 2010; Deshpande et al. 2010; Martuzzi et al. 2010; Vincent et al. 2007; Moeller et al. 2009; Wang et al. 2011; Liang et al. 2013; Scott et al. 2014; Hudetz et al. 2015; Liu et al. 2011a, b, 2013b) have reported that AIU is associated with a disruption of FC within the thalamocortical and frontoparietal networks. To replicate this finding, we examined the absolute FC changes between ROIs in the thalamus, primary sensory-motor, parietal, and prefrontal cortices (see Table 1 for the list of ROIs) between the awake state and an unconscious state (3% isoflurane) using the conventional subtraction method. Figure 3 shows connections with significantly decreased (blue lines) and increased (red lines) FC within the thalamocortical and frontoparietal networks (in the axial and coronal views, two-sample *t* test, $p<0.05$, False Discovery Rate corrected). The data indicate that a large number of connections within these two networks were compromised during AIU. This result confirms the findings reported in the literature. Notably, since we used a volume coil, which contained relatively homogeneous sensitivity, we did not expect significant spatial bias in FC changes detected.

Table 1 Brain regions of interest in the thalamus, primary sensory-motor, parietal, and prefrontal cortices

Thalamus	Primary sensory cortex	Parietal cortex	Prefrontal cortex
Anterior nuclei, dorsal thalamus	Auditory areas	Ectorhinal area	Agranular insular area
Lateral geniculate complex	Gustatory area	Parietal region	Anterior cingulate area
Lateral nuclei, dorsal thalamus	Primary somatomotor area	Retrosplenial area	Infralimbic area
Medial geniculate complex	Primary somatosensory area	Supplemental somatosensory area	Orbital area
Medial nuclei, dorsal thalamus	Secondary somatomotor area	Ventral temporal association areas	Prelimbic area
Midline group, dorsal thalamus	Visceral area		
Reticular nucleus thalamus	Visual areas		
Ventral nuclei, dorsal thalamus			

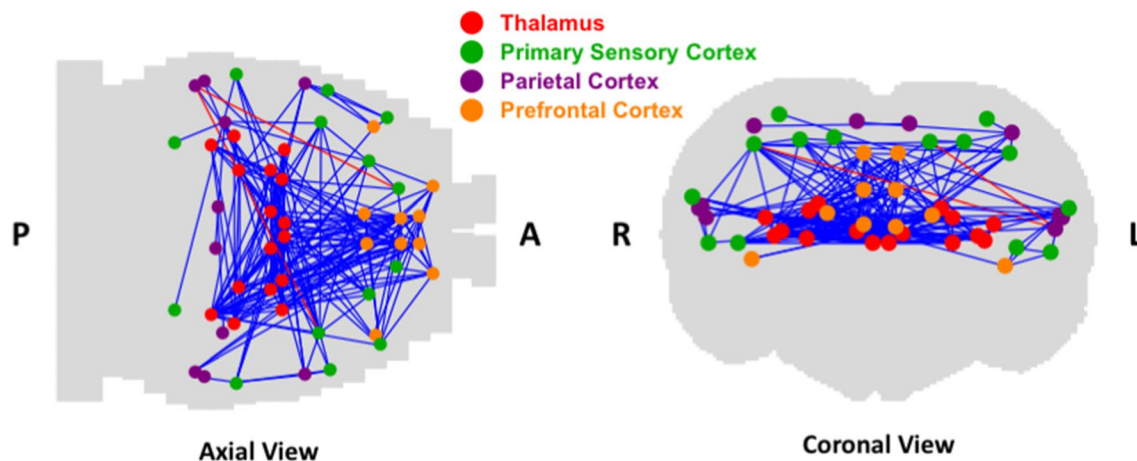


Fig. 3 Significant Δ FC within the thalamocortical and frontoparietal networks at an unconscious state. Significantly decreased (*blue lines*) and increased (*red lines*) FC between ROIs (listed in Table 1) in the thalamus, primary sensory, parietal, and prefrontal cortices (color-

coded) from the awake state to 3.0% isoflurane displayed in the axial and coronal views of the rat brain (*t* test, $p < 0.05$, False Discovery Rate corrected). *P* posterior, *A* anterior, *R* right, *L* left

Δ FC during AIU was proportionally dependent on the FC strength at the awake state

We further examined anesthetic-induced FC changes across the whole brain. The brain was parcellated into 134 unilateral ROIs. For each dose, we calculated the Δ FC between every two ROIs relative to the awake state (134 unilateral ROIs, 8911 connections in total). Intriguingly, this Δ FC *highly depended on the corresponding FC strength at the awake state across all connections in the brain* (Fig. 4a, $r = -0.41, -0.49, -0.62, -0.60, -0.81$, for isoflurane concentrations of 0.5, 1.0, 1.5, 2.0, and 3.0%, respectively, $p < 10^{-200}$ for all concentrations). In addition, this dependency became stronger at higher doses of isoflurane, reflected by increasingly negative correlations between Δ FC and the FC strength at the awake state when the isoflurane dose increased. The difference in these correlations was statistically significant across doses (one-way ANOVA, $F(4,120) = 5.95$, $p = 0.0002$, Fig. 4c). Furthermore, this dependency existed regardless of the spatial scale as evidenced by consistent results obtained for all connections between every pair of brain voxels (Fig. 4b, ~ 6000 voxels, ~ 18 million connections, $r = -0.60, -0.64, -0.71, -0.72$, and -0.79 for isoflurane concentrations of 0.5, 1.0, 1.5, 2.0, and 3.0%, respectively, $p < 10^{-200}$ for all concentrations). The difference in these correlations was also statistically significant across doses for the voxel-wise analysis (one-way ANOVA, $F(4,120) = 3.32$, $p = 0.01$, Fig. 4d).

The brain underwent widespread disruption in information exchange during isoflurane-induced unconsciousness

To investigate the information exchange loss/gain at a local brain region during AIU, for each connection (between two voxels or two ROIs), we first normalized Δ FC at each anesthetized condition to the corresponding FC strength at the awake state (referred to as normalized Δ FC). For each brain voxel (or ROI), we then averaged its normalized Δ FC with all other brain voxels (or ROIs), and this averaged normalized Δ FC was used as a measure of information exchange loss/gain for this voxel (or ROI) (Olde Dubbelink et al. 2013). Figure 5 shows voxel-wise histograms (A) and spatial distributions (B) of the averaged normalized Δ FC for all isoflurane doses. Strikingly, all voxels exhibited negative values for this measure for all doses, indicating an exclusive reduction of information exchange under anesthesia.

Figure 5a also demonstrates that, relative to the awake state, the overall FC strength across the brain was reduced by as much as $\sim 80\%$ (i.e., normalized Δ FC < -0.8) after the animal lost consciousness (i.e., isoflurane dose $\geq 1.5\%$). If the vast majority of meaningful information processing throughout the brain was lost during AIU, it can be predicted that the randomness of spontaneously fluctuating neural activity and rsfMRI signal should significantly increase (Barttfeld et al. 2015). To test this hypothesis, we calculated Shannon entropy and mutual information of the BOLD signal at each condition. Here, entropy, which

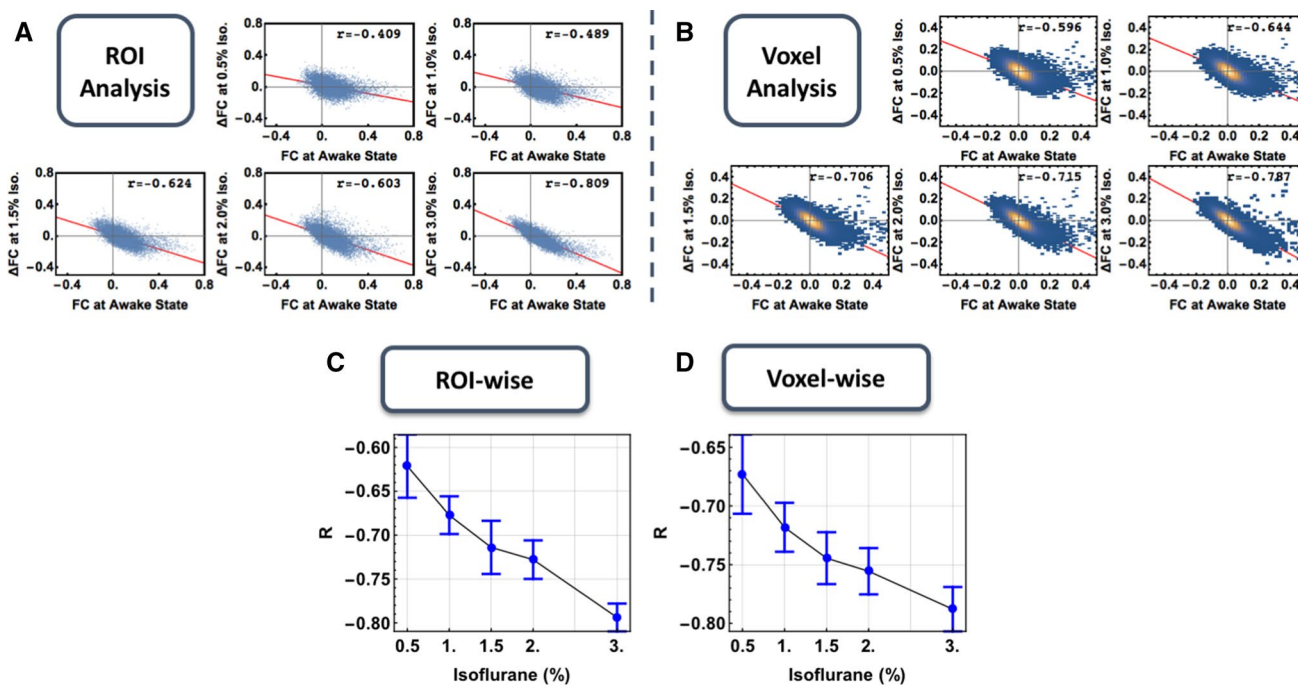


Fig. 4 Δ FC is dependent on the FC strength at the awake state. Scatter plots of **a** ROI-based and **b** voxel-based analyses show that the dependency between Δ FC at anesthetized states and the FC strength at the awake state becomes stronger at higher doses of isoflurane. Correlation coefficient (r) between Δ FC and FC_{awake} as a function of

isoflurane concentration in both ROI- (**c**) and voxel-wise (**d**) analyses. The difference in these correlations is statistically significant across doses for both the ROI (one-way ANOVA, $F(4,120)=5.95$, $p=0.0002$) and the voxel-wise (one-way ANOVA, $F(4,120)=3.32$, $p=0.01$) analyses. Bars SEM

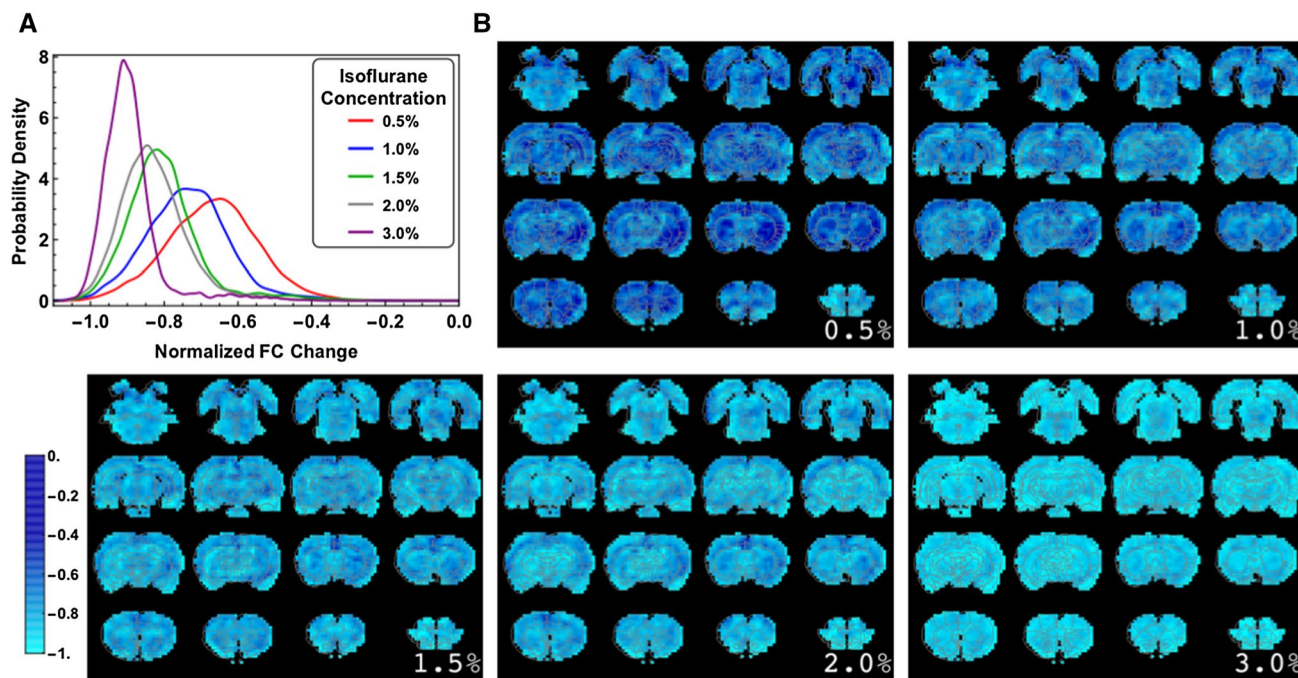


Fig. 5 Distributions and voxel-wise maps of the averaged normalized Δ FC at each dose of isoflurane. **a** Histograms show that all voxels exhibited negative *normalized* Δ FC for all doses, indicating an exclu-

sive reduction of information exchange under anesthesia. **b** Maps reveal a fairly uniform reduction in normalized FC once animals lost consciousness (isoflurane concentration $\geq 1.5\%$)

provided a measure of randomness in the BOLD signal, characterized the amount of information that the signal contains, and mutual information described the amount of information that was exchanged. Figure 6a shows that the entropy of the BOLD time series increased with increasing isoflurane concentration and remained stable beyond the point the animal first lost consciousness (1.5% isoflurane). This entropy change was statistically significant across doses (one-way ANOVA, $F(5,144)=4.99$, $p=0.0003$). Notably, the entropy values during unconsciousness approach that of random noise (average entropy value of simulated random noise=2.01). Conversely, mutual information between brain regions gradually declined as the level of consciousness decreased (Fig. 6b). This change in mutual information was also statistically significant across doses (one-way ANOVA, $F(5,144)=6.20$, $p=0.0003$). Taken together, these data again support that meaningful information exchange was significantly disrupted across the whole brain during isoflurane-induced unconsciousness.

Global reduction in information exchange was not agent specific

To examine whether the global pattern of information exchange disruption during isoflurane-induced unconsciousness was agent-dependent, and also to rule out the possibility that the change we observed resulted from the vascular effects of isoflurane (Ori et al. 1986; Lenz et al. 1998; Maekawa et al. 1986; Alkire et al. 1997), we further investigated FC changes during dexmedetomidine-induced unconsciousness. Dexmedetomidine is an alpha-2-adrenoceptor agonist with virtually no vascular effect, and its molecular action is distinct from isoflurane (Nelson et al. 2003). We acquired rsfMRI scans at both the awake and unconscious states induced by subcutaneous injection of a bolus of 0.05 mg/kg of dexmedetomidine (Angel 1993; Nelson et al. 2003; Pawela et al. 2008; Zhao et al. 2008; Weber et al. 2006), followed by a continuous infusion of dexmedetomidine (0.1 mg/kg/h) initiated 15 min after the bolus injection to maintain unconsciousness (Angel 1993;

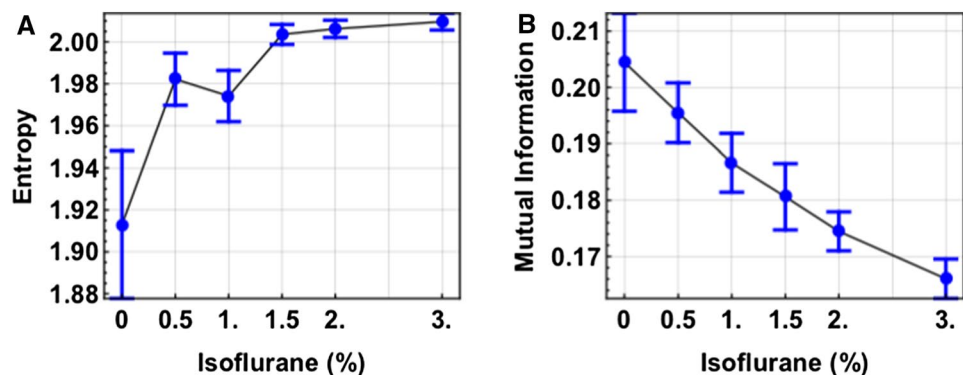
Nelson et al. 2003; Pawela et al. 2008; Zhao et al. 2008; Weber et al. 2006). The dose selected was strong enough to abolish the righting reflex in all animals ($n=6$). rsfMRI data were analyzed in the same way as above.

Similar to isoflurane-induced unconsciousness, we observed a strong dependency between ΔFC at the dexmedetomidine-induced unconscious state and the FC strength at the awake state across all connections (Fig. 7a, ROI-wise analysis, $r=-0.74$, $p < 10^{-200}$; Fig. 7b, voxel-wise analysis: $r=-0.72$, $p < 10^{-200}$). Consistent results between ROI- and voxel-wise analyses indicate that information exchange loss was independent of the spatial scale under dexmedetomidine. Also similar to isoflurane-induced unconscious states, the overall FC strength across the brain was reduced by as much as ~85% (i.e., normalized $\Delta FC < -0.85$) during dexmedetomidine-induced unconsciousness (Fig. 7c), and this information exchange loss was ubiquitous across the brain (Fig. 7d). Moreover, like isoflurane-induced unconsciousness, we observed an increase in the entropy (Fig. 7e) and a decrease in mutual information (Fig. 7f) during dexmedetomidine-induced unconsciousness. Collectively, these data suggest that the global disruption of information exchange is not agent specific and may be an agent-invariant mechanism of AIU.

Discussion

In the present study, we investigated the spatial pattern of anesthetic-induced FC changes across the whole brain in rats. We found that the ΔFC at anesthetized states proportionally depended on the FC strength at the awake state. This dependency became stronger at higher doses of isoflurane and persisted regardless of the spatial scale. To further investigate the regional distribution of information exchange loss/gain during AIU, for each voxel (or ROI), we averaged its normalized ΔFC with all other voxels (or ROIs). This analysis indicates that the brain exhibited exclusive reduction in this averaged normalized FC during unconsciousness, reflecting the brain's diminished capacity

Fig. 6 Entropy and mutual information at each isoflurane dose. **a** The entropy of the BOLD signal increases to a value corresponding to random noise (~2.0), and **b** mutual information appreciably decreases as anesthetic depth increases. Bars SEM across rats.



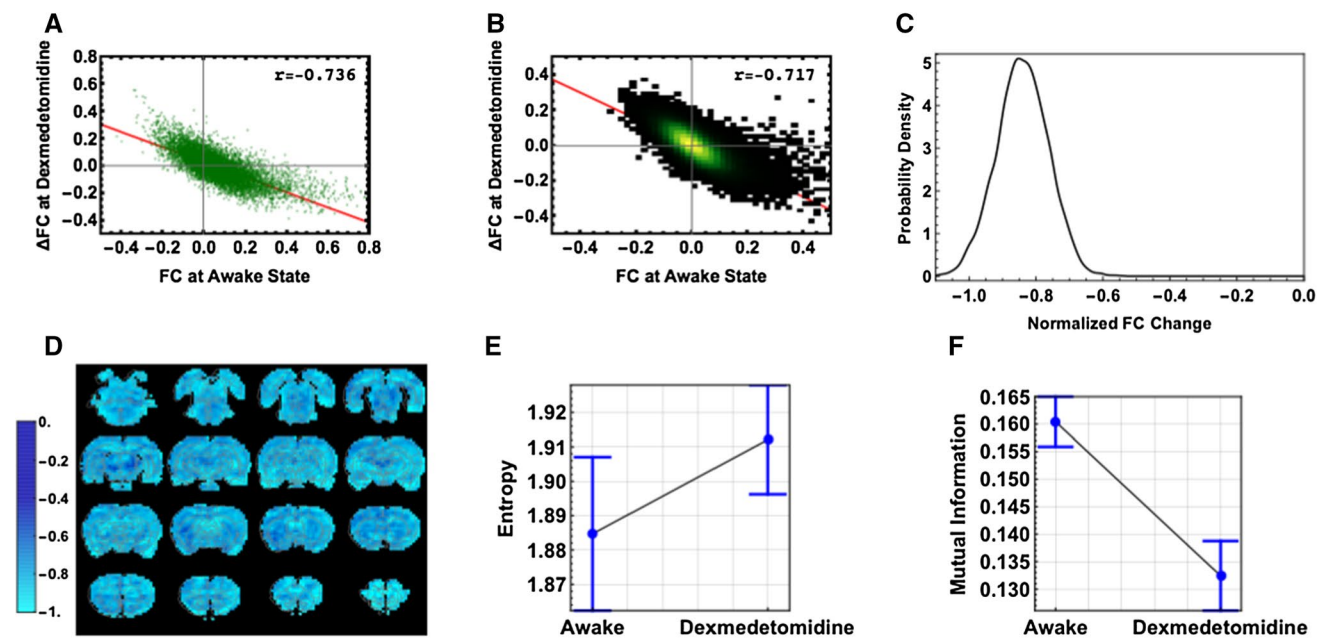


Fig. 7 FC changes during dexmedetomidine-induced unconsciousness. ΔFC at the anesthetized state is dependent on the FC strength at the awake state for both ROI- (a) and voxel-based (b) analyses. The distribution (c) and voxel-wise map (d) of the normalized ΔFC dur-

ing dexmedetomidine-induced unconsciousness. The entropy (e) and mutual information (f) of the BOLD signal at the awake state and during dexmedetomidine administration. *Bars* SEM

to exchange meaningful information between regions. To further support this notion, we demonstrate that after LOC, the entropy of the BOLD signal approached a level comparable to random noise and the mutual information showed a marked reduction. Taken together, these results indicate that during AIU, information exchange is significantly disrupted across the whole brain.

Anesthesia has a global, rather than local, impact on the brain

Since the brain is a highly inter-connected system in which *global* coordination is critical for effective information processing (Moon et al. 2015), and also considering that most anesthetic agents affect neurotransmitters ubiquitously (Franks 2008), it is reasonable to hypothesize that anesthesia has a global impact on brain functioning. To test this hypothesis, a technique that allows whole-brain networks to be studied at different consciousness levels is needed. rsfMRI is ideal for this purpose given its features of global field of view, superb spatial resolution, and high sensitivity to dynamic changes. In addition, rsfMRI allows us to quantify functional communication that occurs when two brain regions generate synchronized neural activity and thus can provide a measure of information exchange at different states. Using this technique, we demonstrate that information exchange loss is exclusive across the brain during AIU.

These results confirm that anesthesia has a global, rather than local, impact on the brain.

We also observed larger decreases of normalized ΔFC at deeper anesthetized depths (Fig. 5). This result indicates that lower consciousness levels might be associated with more global information exchange loss and suggests that rsfMRI may provide a biomarker to measure the consciousness level. Interestingly, based on imaging data, we conjecture that the consciousness level in dexmedetomidine-anesthetized rats was lower than rats under 0.5 and 1% isoflurane as the FC reduction in dexmedetomidine-anesthetized rats in general was greater than rats under 0.5 and 1% isoflurane. This speculation can be supported by their LORR data. No animals lost righting reflex at 0.5 or 1% isoflurane, while all animals lost righting reflex at the dose of dexmedetomidine used, suggesting that the consciousness level in dexmedetomidine-anesthetized rats was lower than rats under 0.5 and 1% isoflurane.

Global information exchange loss is agent independent

Our data provide important evidence that the global reduction of information exchange during AIU might be independent of the anesthetic agent used, as we show consistent findings in rats anesthetized by dexmedetomidine—an anesthetic agent with a drastically different molecular mechanism from isoflurane. Isoflurane is a halogenated ether that affects both neural and vascular

substrates (Angel 1993), whereas dexmedetomidine is an alpha-2-adrenoceptor agonist with virtually no vascular effect. Similar patterns of information exchange loss between dexmedetomidine- and isoflurane-induced unconsciousness can rule out the possibility that the FC changes we observed originated from the vasodilating effect of isoflurane (Lenz et al. 1998). More importantly, these results suggest that global information exchange loss may be a common phenomenon across various anesthetic agents. This notion is supported by a recent study demonstrating disruption of corticocortical information transfer during ketamine anesthesia (Schroeder et al. 2016) and report of breakdown of FC during propofol-induced LOC (Boly et al. 2012; Liu et al. 2013a). Notably, since we did not measure rsfMRI data during unconsciousness of other origins (e.g., slow wave sleep, coma, minimal conscious state, and vegetative state), it might be premature to generalize this conclusion to unconsciousness of all origins. Future studies can extend our analysis to more anesthetic agents and to alternative forms of unconsciousness.

Global reduction of information exchange is associated with increased randomness of spontaneous brain activity

If information exchange indeed diminishes across the whole brain during AIU, one would predict that meaningful information processing would be significantly compromised, giving rise to more random spontaneous neural activity. This notion is supported by our findings that, as the anesthetic depth increases, the entropy of the rsfMRI signal increased to a value comparable to random noise (~ 2.0), and mutual information decreased appreciably. These results are consistent with an independent study reporting that, at a higher isoflurane concentration (2.9%), the temporal scaling characteristics of rsfMRI signal, measured by Hurst exponent (H), approached those of Gaussian white noise ($H=0.5$), in contrast to lower isoflurane concentrations (0.5–1%, $H>0.5$) (Wang et al. 2011). They also agree with a recent study, in which Barttfeld and colleagues (2015) demonstrated that FC at unconscious states arises from “a semi-random circulation of spontaneous neural activity along fixed anatomical routes,” producing a single-brain connectivity pattern dictated by the structure of anatomical connectivity (Barttfeld et al. 2015). These results collectively suggest that global reduction of information exchange is associated with increased randomness of spontaneous brain activity.

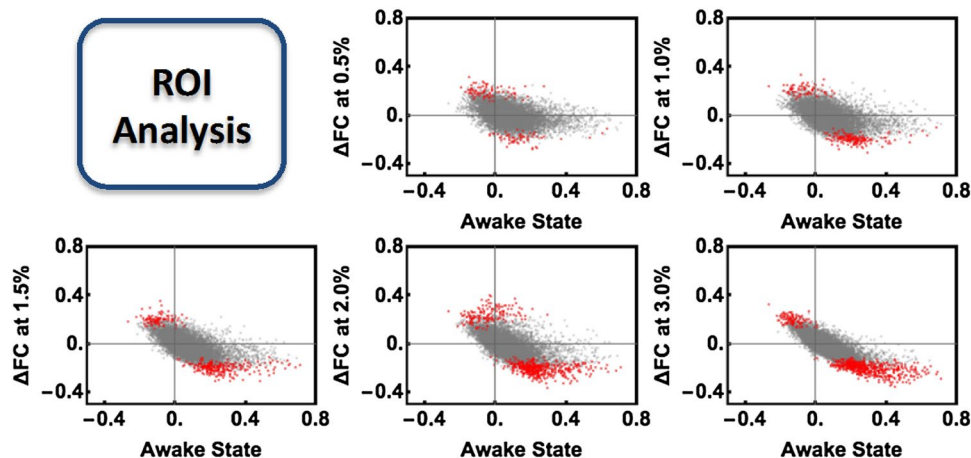
Normalized FC changes can reveal subtle FC differences

The relatively uniform reduction of FC across the whole brain during AIU appears to contradict many neuroimaging studies that highlight specific brain regions/circuits that are particularly sensitive to anesthesia (e.g., thalamocortical and frontoparietal networks). However, this apparent contradiction is most likely attributed to differences in data analysis methods and interpretation of results. Previous neuroimaging studies have relied on the subtraction method that statistically compares FC strength between the awake and anesthetized states (Barttfeld et al. 2015) or between different anesthetic depths (Hutchison et al. 2014; Vincent et al. 2007). As a result, these studies tend to highlight regions/networks that exhibit large FC changes. Indeed, using the conventional subtraction method, our data showed significantly reduced FC in the thalamocortical and frontoparietal networks as well (Fig. 3). Nevertheless, we also found that, in general, connections with stronger FC in the awake state tend to exhibit larger ΔFC during AIU (Fig. 4). Therefore, to reveal more subtle FC changes, and also to account for the influence of the FC strength in the awake state, we normalized ΔFC at each anesthetic depth to the FC strength at the awake state. Consequently, this analysis provides a global, holistic perspective of whole-brain connectivity changes during AIU. To demonstrate that the conventional subtraction method would reveal apparently different results, we identified connections with significant ΔFC (red dots in Fig. 8, $p<0.005$, uncorrected). It is clear from this figure that with the use of the subtraction method, a specific set of connections was highlighted, while the global-scale proportional relationship between ΔFC and FC_{awake} revealed in the present study would no longer be as pronounced. On the other hand, our analysis suggests that the effects of anesthetics go beyond thalamocortical and frontoparietal networks, and can reduce the information exchange across the whole brain. Such results will have important implication for our understanding of system-level mechanisms underlying AIU.

Advantages and limitations

One major advantage of the present study is the utilization of the awake animal imaging approach (Liang et al. 2011; Zhang et al. 2010). Animal fMRI experiments typically rely on anesthesia to immobilize animals, which confounds the effects of the anesthetic agents being studied. Consequently, without the ‘ground truth’ of brain activity/connectivity at the awake state, it is virtually impossible to parcel out the specific effects of an anesthetic agent on global-brain networks, and thus, it is difficult to fundamentally decipher

Fig. 8 Scatter plots illustrating the relationship between ΔFC (between pairs of ROIs) and the strength of the FC at the awake state for each dose of isoflurane. The red points represent the connections that exhibit significant ΔFC using the conventional subtraction method (two-sample t test, $p < 0.005$, uncorrected)



system-level mechanisms underpinning AIU. This obstacle has been overcome in the present study, in which rsfMRI data of awake animals were collected and used as the reference point to determine the whole-brain FC changes during various unconscious conditions.

One potential limitation is the higher motion level in the rsfMRI data at the awake state compared to anesthetized states (averaged volume-to-volume displacement = 0.148 mm for the awake state and 0.066 mm for all anesthetized states). However, the disparity in motion between different consciousness levels cannot explain the relatively uniform FC reduction that we observed during AIU. First, it is unlikely that larger motion at the awake state can result in uniformly stronger FC (Van Dijk et al. 2012). Second, in our data preprocessing, we used a 16-parameter nuisance regression approach, which has been shown to be very effective for removing motion-related artifacts in FC calculations (Power et al. 2015). More importantly, we identified a subset of data ($n=7$) that exhibited minimal movement during the awake state (averaged volume-to-volume displacement = 0.068 mm at the awake state), and

this motion level was similar to that at anesthetized states (one-way ANOVA: $F(4,36) = 2.08$, $p = 0.1$). Figure 9 shows that the pattern of information exchange loss obtained from this subset of data is almost identical to that from the full dataset, suggesting that our results cannot be attributed to difference in motion.

Summary

Overall, the present study provides compelling neuroimaging evidence showing a global reduction of FC during AIU, which reflects the disruption of meaningful information exchange across the whole brain. This notion is bolstered by the increase in entropy of the BOLD signal and a marked reduction in mutual information during unconsciousness. Because this pattern was evident during unconsciousness induced by two distinct anesthetic agents, we suspect that this mechanism may be agent invariant. These findings allude to a common system-level mechanism of AIU, in

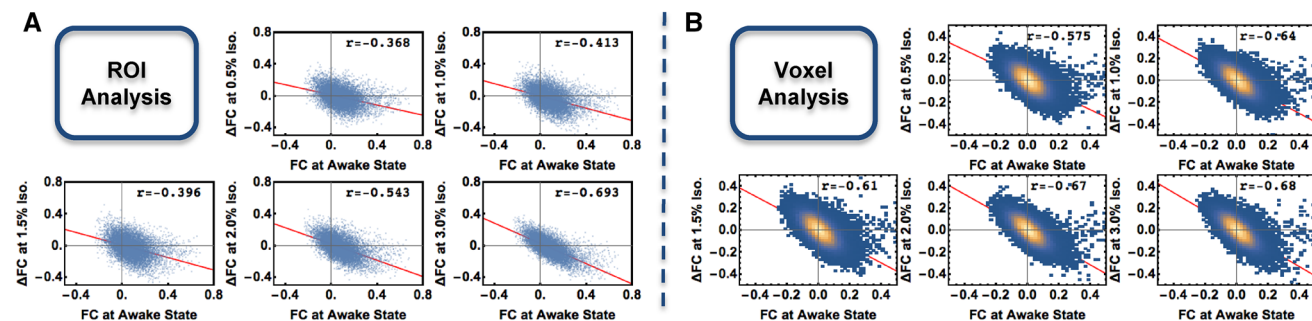


Fig. 9 **a** ROI- and **b** voxel-based analyses of a subset of data that exhibited minimal movement during the awake state. In this subset of data, the motion levels of the awake and anesthetized states are comparable (one-way ANOVA: $F(4,36) = 2.08$, $p = 0.1$). Almost identical

results obtained in the subset suggest that our findings are not attributed to differences in the motion level across different consciousness levels

which the information exchange capacity is affected in all brain regions/networks to a similar degree.

The global reduction of FC associated with anesthetized states might serve as a biomarker for unconsciousness that could be utilized in a clinical setting. Such a biomarker would effectively reduce anesthesia-related complications during surgical procedures. Broadly, this research yields novel insight that contributes to our scientific understanding of consciousness with the potential of uncovering fundamental features of this phenomenon. Such information would be especially valuable to individuals suffering from disorders of consciousness that are non-communicative and non-responsive (e.g., Lock-in Syndrome), as its translation into a clinical context would facilitate both diagnosis and prognosis of these conditions.

Acknowledgements We would like to thank Dr. Pablo Perez for his technical support and Ms Lilith Antinori for editing the manuscript. The work was supported by the National Institutes of Health, Grant Numbers R01MH098003 (PI: Nanyin Zhang, PhD) from the National Institute of Mental Health and R01NS085200 (PI: Nanyin Zhang, PhD) from the National Institute of Neurological Disorders and Stroke.

References

- Alkire MT, Haier RJ, Shah NK, Anderson CT (1997) Positron emission tomography study of regional cerebral metabolism in humans during isoflurane anesthesia. *Anesthesiology* 86(3):549–557
- Alkire MT, Haier RJ, Fallon JH (2000) Toward a unified theory of narcosis: brain imaging evidence for a thalamocortical switch as the neurophysiologic basis of anesthetic-induced unconsciousness. *Conscious Cogn* 9(3):370–386. doi:10.1006/ccog.1999.0423
- Alkire MT, Hudetz AG, Tononi G (2008) Consciousness and anesthesia. *Science* 322(5903):876–880. doi:10.1126/science.1149213
- Angel A (1991) The G. L. Brown lecture. *Adventures in anaesthesia*. *Exp Physiol* 76(1):1–38
- Angel A (1993) Central neuronal pathways and the process of anaesthesia. *Br J Anaesth* 71(1):148–163
- Barttfeld P, Uhrig L, Sitt JD, Sigman M, Jarraya B, Dehaene S (2015) Signature of consciousness in the dynamics of resting-state brain activity. *Proc Natl Acad Sci USA* 112(3):887–892. doi:10.1073/pnas.1418031112
- Biswal B, Yetkin FZ, Haughton VM, Hyde JS (1995) Functional connectivity in the motor cortex of resting human brain using echoplanar MRI. *Magn Reson Med* 34(4):537–541
- Boly M, Moran R, Murphy M, Boveroux P, Bruno MA, Noirhomme Q, Ledoux D, Bonhomme V, Brichant JF, Tononi G, Laureys S, Friston K (2012) Connectivity changes underlying spectral EEG changes during propofol-induced loss of consciousness. *J Neurosci* 32(20):7082–7090. doi:10.1523/JNEUROSCI.3769-11.2012
- Boveroux P, Vanhauwenhuysse A, Bruno MA, Noirhomme Q, Lauwick S, Luxen A, Degueldre C, Plenevaux A, Schnakers C, Phillips C, Brichant JF, Bonhomme V, Maquet P, Greicius MD, Laureys S, Boly M (2010) Breakdown of within- and between-network resting state functional magnetic resonance imaging connectivity during propofol-induced loss of consciousness. *Anesthesiology* 113(5):1038–1053. doi:10.1097/ALN.0b013e3181f697f5
- Breshears JD, Roland JL, Sharma M, Gaona CM, Freudenburg ZV, Tempelhoff R, Avidan MS, Leuthardt EC (2010) Stable and dynamic cortical electrophysiology of induction and emergence with propofol anesthesia. *Proc Natl Acad Sci USA* 107(49):21170–21175. doi:10.1073/pnas.1011949107
- Brown EN, Lydic R, Schiff ND (2010) General anesthesia, sleep, and coma. *N Engl J Med* 363(27):2638–2650. doi:10.1056/NEJMr0808281
- Deshpande G, Kerssens C, Sebel PS, Hu X (2010) Altered local coherence in the default mode network due to sevoflurane anesthesia. *Brain Res* 1318:110–121. doi:10.1016/j.brainres.2009.12.075
- Franks NP (2008) General anaesthesia: from molecular targets to neuronal pathways of sleep and arousal. *Nat Rev Neurosci* 9(5):370–386. doi:10.1038/nrn2372
- Hudetz AG, Vizuete JA, Pillay S (2011) Differential effects of isoflurane on high-frequency and low-frequency gamma oscillations in the cerebral cortex and hippocampus in freely moving rats. *Anesthesiology* 114(3):588–595. doi:10.1097/ALN.0b013e31820ad3f9
- Hudetz AG, Liu X, Pillay S (2015) Dynamic repertoire of intrinsic brain states is reduced in propofol-induced unconsciousness. *Brain connectivity* 5(1):10–22. doi:10.1089/brain.2014.0230
- Hutchison RM, Hutchison M, Manning KY, Menon RS, Everling S (2014) Isoflurane induces dose-dependent alterations in the cortical connectivity profiles and dynamic properties of the brain's functional architecture. *Hum Brain Mapp* 35(12):5754–5775. doi:10.1002/hbm.22583
- Imas OA, Ropella KM, Ward BD, Wood JD, Hudetz AG (2005) Volatile anesthetics disrupt frontal-posterior recurrent information transfer at gamma frequencies in rat. *Neurosci Lett* 387(3):145–150. doi:10.1016/j.neulet.2005.06.018
- Lee U, Mashour GA, Kim S, Noh GJ, Choi BM (2009) Propofol induction reduces the capacity for neural information integration: implications for the mechanism of consciousness and general anesthesia. *Conscious Cogn* 18(1):56–64. doi:10.1016/j.concog.2008.10.005
- Lee U, Ku S, Noh G, Baek S, Choi B, Mashour GA (2013) Disruption of frontal-parietal communication by ketamine, propofol, and sevoflurane. *Anesthesiology* 118(6):1264–1275. doi:10.1097/ALN.0b013e31829103f5
- Lenz C, Rebel A, van Ackern K, Kuschinsky W, Waschke KF (1998) Local cerebral blood flow, local cerebral glucose utilization, and flow-metabolism coupling during sevoflurane versus isoflurane anesthesia in rats. *Anesthesiology* 89(6):1480–1488
- Liang Z, King J, Zhang N (2011) Uncovering intrinsic connective architecture of functional networks in awake rat brain. *J Neurosci* 31(10):3776–3783
- Liang Z, King J, Zhang N (2012a) Anticorrelated resting-state functional connectivity in awake rat brain. *Neuroimage* 59(2):1190–1199. doi:10.1016/j.neuroimage.2011.08.009
- Liang Z, King J, Zhang N (2012b) Intrinsic organization of the anesthetized brain. *J Neurosci* 32(30):10183–10191. doi:10.1523/JNEUROSCI.1020-12.2012
- Liang Z, Li T, King J, Zhang N (2013) Mapping thalamocortical networks in rat brain using resting-state functional connectivity. *Neuroimage* 83:237–244. doi:10.1016/j.neuroimage.2013.06.029
- Liang Z, King J, Zhang N (2014) Neuroplasticity to a single-episode traumatic stress revealed by resting-state fMRI in awake rats. *Neuroimage* 103:485–491. doi:10.1016/j.neuroimage.2014.08.050
- Liang Z, Liu X, Zhang N (2015a) Dynamic resting state functional connectivity in awake and anesthetized rodents. *Neuroimage* 104:89–99. doi:10.1016/j.neuroimage.2014.10.013
- Liang Z, Watson GD, Alloway KD, Lee G, Neuberger T, Zhang N (2015b) Mapping the functional network of medial prefrontal cortex by combining optogenetics and fMRI in

- awake rats. *Neuroimage* 117:114–123. doi:[10.1016/j.neuroimage.2015.05.036](https://doi.org/10.1016/j.neuroimage.2015.05.036)
- Liu X, Zhu XH, Zhang Y, Chen W (2011) Neural origin of spontaneous hemodynamic fluctuations in rats under burst-suppression anesthesia condition. *Cereb Cortex* 21(2):374–384. doi:[10.1093/cercor/bhq105](https://doi.org/10.1093/cercor/bhq105)
- Liu X, Pillay S, Li R, Vizuete JA, Pechman KR, Schmainda KM, Hudetz AG (2013a) Multiphasic modification of intrinsic functional connectivity of the rat brain during increasing levels of propofol. *Neuroimage* 83:581–592. doi:[10.1016/j.neuroimage.2013.07.003](https://doi.org/10.1016/j.neuroimage.2013.07.003)
- Liu X, Zhu XH, Zhang Y, Chen W (2013b) The change of functional connectivity specificity in rats under various anesthesia levels and its neural origin. *Brain Topogr* 26(3):363–377. doi:[10.1007/s10548-012-0267-5](https://doi.org/10.1007/s10548-012-0267-5)
- Ma Y, Hamilton C, Zhang N (2016) Dynamic connectivity patterns in conscious and unconscious brain. *Brain Connectivity*. doi:[10.1089/brain.2016.0464](https://doi.org/10.1089/brain.2016.0464)
- MacIver MB, Bland BH (2014) Chaos analysis of EEG during isoflurane-induced loss of righting in rats. *Front Syst Neurosci* 8:203. doi:[10.3389/fnsys.2014.00203](https://doi.org/10.3389/fnsys.2014.00203)
- Maekawa T, Tommasino C, Shapiro HM, Keifer-Goodman J, Kohlenberger RW (1986) Local cerebral blood flow and glucose utilization during isoflurane anesthesia in the rat. *Anesthesiology* 65(2):144–151
- Martuzzi R, Ramani R, Qiu M, Rajeevan N, Constable RT (2010) Functional connectivity and alterations in baseline brain state in humans. *Neuroimage* 49(1):823–834. doi:[10.1016/j.neuroimage.2009.07.028](https://doi.org/10.1016/j.neuroimage.2009.07.028)
- Mashour GA (2014) Top-down mechanisms of anesthetic-induced unconsciousness. *Front Syst Neurosci* 8:115. doi:[10.3389/fnsys.2014.00115](https://doi.org/10.3389/fnsys.2014.00115)
- Mashour GA, Alkire MT (2013) Evolution of consciousness: phylogeny, ontogeny, and emergence from general anesthesia. *Proc Natl Acad Sci USA* 110(Suppl 2):10357–10364. doi:[10.1073/pnas.1301188110](https://doi.org/10.1073/pnas.1301188110)
- Moeller S, Nallasamy N, Tsao DY, Freiwald WA (2009) Functional connectivity of the macaque brain across stimulus and arousal states. *J Neurosci* 29(18):5897–5909. doi:[10.1523/JNEUROSCI.0220-09.2009](https://doi.org/10.1523/JNEUROSCI.0220-09.2009)
- Moon JY, Lee U, Blain-Moraes S, Mashour GA (2015) General relationship of global topology, local dynamics, and directionality in large-scale brain networks. *PLoS Comput Biol* 11(4):e1004225. doi:[10.1371/journal.pcbi.1004225](https://doi.org/10.1371/journal.pcbi.1004225)
- Murphy K, Birn RM, Handwerker DA, Jones TB, Bandettini PA (2009) The impact of global signal regression on resting state correlations: are anti-correlated networks introduced? *Neuroimage* 44(3):893–905
- Nelson LE, Lu J, Guo T, Saper CB, Franks NP, Maze M (2003) The alpha2-adrenoceptor agonist dexmedetomidine converges on an endogenous sleep-promoting pathway to exert its sedative effects. *Anesthesiology* 98(2):428–436
- Olde Dubbelink KT, Stoffers D, Deijen JB, Twisk JW, Stam CJ, Hillebrand A, Berendse HW (2013) Resting-state functional connectivity as a marker of disease progression in Parkinson's disease: a longitudinal MEG study. *Neuroimage Clin* 2:612–619. doi:[10.1016/j.nicl.2013.04.003](https://doi.org/10.1016/j.nicl.2013.04.003)
- Ori C, Dam M, Pizzolato G, Battistin L, Giron G (1986) Effects of isoflurane anesthesia on local cerebral glucose utilization in the rat. *Anesthesiology* 65(2):152–156
- Pawela CP, Biswal BB, Cho YR, Kao DS, Li R, Jones SR, Schulte ML, Matloub HS, Hudetz AG, Hyde JS (2008) Resting-state functional connectivity of the rat brain. *Magn Reson Med* 59(5):1021–1029
- Power JD, Schlaggar BL, Petersen SE (2015) Recent progress and outstanding issues in motion correction in resting state fMRI. *Neuroimage* 105:536–551. doi:[10.1016/j.neuroimage.2014.10.044](https://doi.org/10.1016/j.neuroimage.2014.10.044)
- Schroeder KE, Irwin ZT, Gaidica M, Bentley JN, Patil PG, Mashour GA, Chestek CA (2016) Disruption of corticocortical information transfer during ketamine anesthesia in the primate brain. *Neuroimage* 134:459–465. doi:[10.1016/j.neuroimage.2016.04.039](https://doi.org/10.1016/j.neuroimage.2016.04.039)
- Scott G, Fagerholm ED, Mutoh H, Leech R, Sharp DJ, Shew WL, Knopfel T (2014) Voltage imaging of waking mouse cortex reveals emergence of critical neuronal dynamics. *J Neurosci* 34(50):16611–16620. doi:[10.1523/JNEUROSCI.3474-14.2014](https://doi.org/10.1523/JNEUROSCI.3474-14.2014)
- Swanson LW (2004) Brain maps: structure of the rat brain. Elsevier
- Tononi G (2004) An information integration theory of consciousness. *BMC Neurosci* 5:42. doi:[10.1186/1471-2202-5-42](https://doi.org/10.1186/1471-2202-5-42)
- Van Dijk KR, Sabuncu MR, Buckner RL (2012) The influence of head motion on intrinsic functional connectivity MRI. *Neuroimage* 59(1):431–438. doi:[10.1016/j.neuroimage.2011.07.044](https://doi.org/10.1016/j.neuroimage.2011.07.044)
- Velly LJ, Rey MF, Bruder NJ, Gouvitsos FA, Witjas T, Regis JM, Peragut JC, Gouin FM (2007) Differential dynamic of action on cortical and subcortical structures of anesthetic agents during induction of anesthesia. *Anesthesiology* 107(2):202–212. doi:[10.1097/01.anes.0000270734.99298.b4](https://doi.org/10.1097/01.anes.0000270734.99298.b4)
- Vincent JL, Patel GH, Fox MD, Snyder AZ, Baker JT, Van Essen DC, Zempel JM, Snyder LH, Corbetta M, Raichle ME (2007) Intrinsic functional architecture in the anaesthetized monkey brain. *Nature* 447(7140):83–86
- Wang K, van Meer MP, van der Marel K, van der Toorn A, Xu L, Liu Y, Viergever MA, Jiang T, Dijkhuizen RM (2011) 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. *NMR Biomed* 24(1):61–67. doi:[10.1002/nbm.1556](https://doi.org/10.1002/nbm.1556)
- Weber R, Ramos-Cabrera P, Wiedermann D, van Camp N, Hoehn M (2006) A fully noninvasive and robust experimental protocol for longitudinal fMRI studies in the rat. *Neuroimage* 29(4):1303–1310. doi:[10.1016/j.neuroimage.2005.08.028](https://doi.org/10.1016/j.neuroimage.2005.08.028)
- White NS, Alkire MT (2003) Impaired thalamocortical connectivity in humans during general-anesthetic-induced unconsciousness. *Neuroimage* 19(2 Pt 1):402–411
- Zhang N, Rane P, Huang W, Liang Z, Kennedy D, Frazier JA, King J (2010) Mapping resting-state brain networks in conscious animals. *J Neurosci Methods* 189(2):186–196
- Zhao F, Zhao T, Zhou L, Wu Q, Hu X (2008) BOLD study of stimulation-induced neural activity and resting-state connectivity in medetomidine-sedated rat. *Neuroimage* 39(1):248–260