INVESTIGATING THE GLOBAL REDUCTION OF INFORMATION EXCHANGE DURING ANESTHETIC-INDUCED UNCONSCIOUSNESS

A Thesis in
Neuroscience

by
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ABSTRACT

During anesthetic-induced unconsciousness (AIU), the brain undergoes a dramatic change in its ability to exchange information between regions. However, the spatial distribution of information exchange loss across the entire brain remains elusive. In this thesis resting-state functional magnetic resonance imaging (rsfMRI) data was acquired in rats during wakefulness and graded depths of anesthesia induced by incrementally increasing the concentration of isoflurane. We found that, regardless of spatial scale, the absolute functional connectivity (FC) change was significantly 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 (i.e. the FC change normalized to the corresponding FC strength at the awake state) exhibited a spatially homogenous reduction across the whole brain particularly after animals lost consciousness, indicating a globally uniform disruption of meaningful information exchange. 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. These findings provide compelling neuroimaging evidence suggesting that the brain undergoes a widespread, uniform disruption in the exchange of meaningful information during AIU, and that this change may represent a common systems-level neural mechanism of AIU.
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Chapter 1

Introduction

Consciousness is a phenomenon that has perplexed scientists and philosophers for centuries. While many would agree that consciousness arises as a function of the brain, it has remained elusive due to its lack of definition and standardized means of measurement. Nevertheless, scientists from diverse fields have attempted to define and measure aspects of brain function associated with consciousness using a variety of techniques including electroencephalography (EEG), electrocorticography (ECog), local field potentials (LFPs), positron emission tomography (PET), and functional magnetic resonance imaging (fMRI). As a result, many frameworks for describing consciousness exist (i.e. the integrated information theory, biological theory, higher-order representationalism, recurrent processing theory, global workspace theory, first order representationalism, etc.) (Laureys et al., 2007; Alkire et al., 2008). One such framework seeks to characterize different "states" of consciousness, each defined by the unique combination of level of arousal and awareness of the environment or self (Figure 1-1) (Laureys et al., 2007). While a precise mechanism of how these two dimensions interact to form a state of consciousness is unknown, it is believed that they are modulated by different functional brain systems.
The level of arousal, or tone of consciousness, is largely maintained by brainstem regions—more specifically, the ascending reticular activating system (aRAS). The first demonstration of this was in 1949 when Moruzzi and Magoun acutely lesioned various regions within the brainstem of cats and observed a loss of consciousness. They concluded that the there was a system, or series of relays, that transmit information beginning in brainstem that ascended up towards the basal regions of diencephalon (Mouruzzi & Magoun, 1949). Now, we are familiar with specific nuclei within the brainstem, such as mesopontine tegmental area (Devor & Zalkind, 2001), that have functions related to respiration (Feldman et al., 2003) and heart rate (Saper et al., 2005). The level of arousal of the CNS is also modulated by brainstem nuclei, periaqueductal grey (PAG) and locus ceruleus, that disperse neurotransmitters, such as dopamine, norepinephrine, and serotonin, across the brain to modulate the tone of the nervous system (Brown et al., 2010).

Figure 1-1: Schematic representing two interacting dimensions that produce various states of consciousness (Laureys et al., 2007).
In contrast, the level of awareness, referred to as the “content” of consciousness, is believed to be mediated by a distinct set of brain areas. Much less is known about these circuits because more variation exists between individuals. To better understand the “content” of consciousness, researchers have operationalized higher-order brain functions such as perceptual binding, planning, decision-making, visual attention, and other forms of cognition (Laureys et al., 2007; Alkire, Hudetz, Tononi, 2008). Interestingly, individuals in a vegetative state (VS) have exhibited an approximate 50% decrease in total brain metabolism, experiencing normal arousal levels, but limited to no awareness of their self or environment (Laureys et al., 2005). These individuals demonstrate preserved brain metabolism in regions associated with arousal (pedunculopontine reticular formation, hypothalamus, and basal forebrain structures), whereas large discrepancies in frontoparietal brain networks are observed (Laureys et al., 2002; 2005). Thalamocortical circuits have also been implicated in conscious awareness, showing that connectivity between intralaminar thalamic nuclei and prefrontal and anterior cingulate cortices is altered during vegetative states, but not after the recovery of consciousness (Laureys, 2000). Thus, it appears that conscious awareness does not occur in a singular brain region, but instead requires the integration of information across globally dispersed brain regions (Alkire et al., 2008).

**Anesthetic-Induced Unconsciousness**

One common strategy to better understand the fundamental properties of consciousness is to study altered forms of consciousness as modulated in pharmacological, physiological or pathological conditions (e.g. anesthesia, coma, sleep, schizophrenia, vegetative state). A state that is particularly useful is anesthetic-induced
unconsciousness (AIU), which can be achieved by altering the level of arousal and/or awareness using anesthetic agents (Brown et al. 2010). Unlike other altered states of consciousness, AIU is temporary and reversible which allows researchers to measure brain function across manipulations such as the loss of and recovery of consciousness (Xie et al., 2011; Hudson et al. 2014). Furthermore, AIU is a convenient strategy because it offers precise experimental control over variables such as anesthetic agent, dosage, and administration.

While anesthetic agents share a common behavioral endpoint (i.e. unconsciousness), they have distinct molecular mechanisms that affect neural and vascular substrate uniquely. Anesthesia is a temporary state of unconsciousness produced when an anesthetic agent significantly depresses the activity of the central nervous system. There are a wide variety of anesthetic agents that generate a depression of the CNS through their own unique cellular and molecular mechanisms (Franks, 2008). Anesthetic agents alter neuronal activity by suppressing glutamatergic transmission and enhancing GABAergic and glycinergic transmission (Brown et al., 2010). Each agent accomplishes this through a unique mechanism and in different regions of the brain. It is believed that isoflurane’s depressant effects on glutamatergic transmission are primarily presynaptic in nature. Isoflurane has been shown to block sodium channels thereby reducing the overall excitability of the cell and decreasing the amount of glutamate released at the synapse (Hemmings, 2009). Unlike isoflurane, dexmedetomidine, an alpha-2-adrenoceptor agonist, produces anesthesia in a manner that resembles natural sleep (Nelson et al., 2003). Dexmedetomidine binds to α2-adrenoceptors in the locus coeruleus (LC), a region known for its role in regulating arousal level through the production and release of norepinephrine (Correa-Sales et al., 1992). Dexmedetomidine activates inwardly rectifying potassium channels, which
facilitate an efflux of potassium, ultimately inhibiting voltage-gated calcium channels and hyperpolarizing the cell (Williams & North, 1985; Williams et al., 1985). The decreased firing rate of these neurons is thought to be a key component in dexmedetomidine’s mechanism of action (Correa-Sales et al., 1992; Nacif-Coelho et al., 1994). Another common anesthetic agent, propofol, acts on GABA_A receptor function in several ways that potentiate GABA-evoked currents (Maclver et al., 1991; Bai et al., 1999; Rudolph et al., 2004; Brown et al., 2010). Specifically, it enhances GABAergic transmission in the cortex and at the inhibitory projections from the preoptic area of the hypothalamus to the arousal centers.

Although the molecular basis of various anesthetic agents is fairly well understood (Franks, 2008; Brown et al., 2010; Rudolph & Antkowiak, 2004), the systems-level mechanisms of AIU remain obscure (Alkire et al., 2008). Accumulating evidence from functional neuroimaging studies have demonstrated the feasibility of measuring changes in systems-level brain function in response to anesthetic agents (Leslie et al., 2000; White & Alkire, 2003). The most commonly used method is blood oxygenated level dependent (BOLD) fMRI, which detects changes in the ratio of oxygenated and deoxygenated hemoglobin in blood (Ogawa et al., 1990). This ratio is altered by changes in blood flow and volume as a result of an increased metabolic demand of active neurons. The BOLD signal has been shown to be tightly coupled to neural activity (Logothetis et al., 2001) and thus, is commonly used as a surrogate measure. In addition, the degree of temporal coherence (measured via correlation) between the BOLD signal time series of individual brain regions (or voxels) has been used as a measure of functional connectivity (FC) (Friston, 1995).
Resting-state Functional Magnetic Resonance Imaging

Resting-state fMRI (rsfMRI) has been widely utilized to investigate the changes of neural circuitries and networks during altered physiological and pathological conditions due to its’ unique features of noninvasiveness, high reliability, and high sensitivity to dynamic changes. rsfMRI is different from the convention task-based fMRI in that it does not involve active stimuli, but instead relies on low-frequency spontaneous fluctuations of the BOLD signal to quantify resting-state functional connectivity (RSFC). Additionally, rsfMRI has a global field of view that allows the whole-brain network to be conveniently imaged (Biswal et al., 1995; Grecius et al., 2003). As a result, rsfMRI is an ideal tool for studying global brain networks during anesthetic-induced unconsciousness (Mashour et al., 2013). Literature has reported that RSFC is correlated with the degree of consciousness from locked-in syndrome, minimally conscious state, vegetative state, to brain death in humans (Boly et al., 2009; Cauda et al., 2009; Vanhaudenhuyse et al., 2010; Laureys & Schiff, 2012).

Furthermore, the use of rsfMRI in animal models during anesthesia have been vast (Vincent et al., 2007; Moeller et al., 2009; Wang et al., 2011; Liu et al., 2011; Williams et al., 2010). Relative to human studies, animal studies of AIU offer several advantages. First, different anesthetic agents can be applied to the same animal to address the question of whether or not anesthetics possess a common systems-level mode of action in producing unconsciousness. Second, anesthetic depths can be easily manipulated. Third, combined with other well-established preclinical tools, animal studies can help link cellular and molecular mechanisms to systems-level changes during AIU. Most animal rsfMRI studies of AIU compare different anesthetic depths without the reference to the awake condition (Vincent et al., 2007; Moeller et al., 2009; Wang et al.,
2011; Liu et al., 2011; Williams et al., 2010). This is because animal fMRI experiments typically rely on anesthesia to immobilize animals, which confounds our investigation of the effects of anesthetic agents (Angel, 1991; Detsch et al., 1999; Vahle-Hinz et al., 2002). Consequently, without an accurate measurement of rsfMRI data at the awake state, it is virtually impossible to parcel out the changes of global brain networks from an awake state to an unconscious state, and thus is unable to fundamentally decipher the neural mechanism underpinning AIU. To resolve this issue, our lab has established an approach to imaging brain network function in fully awake animals. In our imaging paradigm, animals’ motion and stress during MRI scanning are minimized by using a routine acclimation procedure. Using this paradigm, we have investigated various aspects of rat brain function in vivo at the awake state (Liang2012a; Liang 2011; Liang 2012b; Liang 2014; 2013; 2015a; Zhang et al., 2010; Liang et al., 2015b). Importantly, our approach enables studies to investigate the dynamic reorganization of the global brain network during the transition from a fully awake state to an unconscious state, and thus can help uncover the systems-level neural mechanisms underlying AIU.

**Investigating the global reduction of information exchange during AIU**

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, Haier, Fallon, 2000; Tononi et al., 2004; Mashour 2013; Mashour 2014; Lee 2013; Liang 2012; Bovoureux 2010; Martuzzi 2010). This notion has been supported by a number of studies in both humans (Bovoureux 2010, Martuzzi 2010; White & Alkire 2003; Deshpande et al., 2010) and animals (Vincent et al., 2007; Moller et al., 2009; Wang et al., 2011), which have indicated that a
disruption of FC specifically within the thalamocortical and frontoparietal networks is essential to AIU (White & Alkire, 2003; Angel, 1991; Velley 2007; Lee et al., 2009; Breshears et al., 2010).

While these 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 clarifying whether anesthetics produce unconsciousness through actions in specific neural networks (e.g. the thalamocortical network) or whether AIU results from a loss of information exchange across the whole brain. To address this issue, it is essential to elucidate changes across whole-brain networks during AIU. The importance of such investigation is also highlighted by the highly inter-connected characteristics of brain organization, in which global coordination is critical for effective information exchange (Liang et al., 2012a; Moon et al., 2015). In addition, most anesthetic agents affect neurotransmitters throughout the entire brain (Franks, 2008). Therefore, unraveling the effects of anesthesia on the whole-brain network can be critical for deciphering the systems-level neural mechanisms of AIU.

In the present thesis, we employed rsfMRI to investigate information exchange across the whole rat brain during wakefulness and graded levels of unconsciousness induced by increasing concentrations of isoflurane (Figure 2-1). Our data revealed that relative to the awake state, the FC change during unconsciousness is uniformly reduced across the whole brain, which indicates that the disruption of information transfer is ubiquitous in the brain. This spatial pattern of information exchange loss was similar during unconsciousness 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.
Chapter 2

Experimental Paradigm & Procedures

This thesis consists of data collected from thirty-seven adult male Long-Evans rats (300-500g) which were housed and maintained on a 12hr light: 12hr dark schedule, and provided access to food and water ad libitum. We obtained approval for the following experiments from the Institutional Animal Care and Use Committee of the Pennsylvania State University.

Acclimation Procedure

Animals were first acclimated to the scanning environment for 7 days to minimize stress and motion during imaging at the awake state (described in King et al., 2005; Liang et al., 2013; Zhang et al., 2010; Liang et al., 2011 & 2012). To do this, a topical analgesic was applied to the jaw and nose regions of the rat 20 minutes before (as well as just prior to) set-up to reduce any potential pain generated by the physical pressure of the head restraint. Each animal was initially sedated by being placed in an induction chamber filled with isoflurane anesthetic (3.5%). Upon unconsciousness, a nose-cone was used to administer isoflurane for the duration of the set-up. The head of the rat was placed in an inner head restraint in which the teeth were situated over a bite-bar (Figure 1-1B). The animal was then placed in an outer head restraint where plastic screws were tightened to fasten the inner and outer head restraints together (Figure 1-1D). A nose-bar was adjusted down onto the nose, fixing it to the bite-bar (Figure 1-1E). The animal's
limbs were bound with surgical tape to protect itself during the procedure and the animal was then secured into a plexiglass body tube (Figure 1-1F). At this point the animal was allowed to return to an awake state. The body tube was placed into a 4-chambered black opaque box where prerecorded MRI sounds were played (Figure 1-1G). The exposure time in the box was incrementally increased by 15 minutes each day up to 60 minutes for days 4, 5, 6 and 7.

Figure 2-1: **Steps in the acclimation procedure.** The progression of the acclimation procedure is delineated in steps A-G.

**MRI Experiments**

For all MRI experiments, rats were briefly anesthetized with isoflurane while they were placed in a head restrainer with a built-in birdcage coil. Isoflurane was discontinued, but the nosecone remained fastened around the animal’s nose
for the duration of the experiment. Imaging began 30 minutes after rats were placed in the scanner while animals were fully awake. Image acquisition was performed on a 7T 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, slice thickness = 1 mm.

rsfMRI data were obtained during the awake state and various anesthetic depths by systematically increasing the concentration of isoflurane from 0% (i.e. awake state) to 3% (0%, 0.5%, 1.0%, 1.5%, 2.0%, 3.0%)—administered via nosecone. rsfMRI data were acquired using a single-shot gradient-echo, echo planar imaging pulse sequence with TR = 1000 ms, TE = 13.78 ms, flip angle = 80°, matrix size = 64 x 64, FOV = 3.2 x 3.2 cm, slice number = 20, 1mm thick slices (in-plane resolution = 500 mm x 500 mm). For each rsfMRI scan, 600 volumes were acquired in 10 minutes. Between each dose, 5 minutes were allowed to ensure the new dosage reached steady state (see Figure 2-2).

Figure 2-2: 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. Before each dose, 5-minute transition periods were provided to allow animals to reach a steady state.

**Behavioral Experiments**

For over a century, the loss of righting reflex (LORR) has been widely used as a surrogate index of unconsciousness in rodents. Researchers have demonstrated a strong correlation between the concentration of various anesthetic agents needed to produce the loss of voluntary movement in rodents and loss of consciousness in humans (unresponsiveness to verbal commands) over five orders of magnitude (Franks, 2008). We assessed the animal’s conscious level when graded concentrations of isoflurane were delivered through a nose cone (0.5%, 1%, 1.5% 2% and 3%) by measuring the LORR. LORR was measured outside of the scanner in a manner that directly mimicked anesthesia administration during the scanning process, allowing us to verify the animals’ vigilance level at each anesthetic dose during rsfMRI data acquisition (Figure 2-2).

In the LORR procedure, each rat began in a restrained, awake state (i.e. 0% isoflurane) and was exposed to isoflurane through a nosecone. We measured the LORR for a single dose delivered to the rat exactly as it would be in the scanner (Figure 2-2): Each scan was 10 minutes with a 5-minute transition time between scans to allow the isoflurane concentration to reach a steady state. Thus, for example, to measure LORR at 1.5% isoflurane, the animal first
received 0.5% isoflurane for 15 minutes, then 1.0% isoflurane for 15 minutes, and finally 1.5% isoflurane for 5 minutes. 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 seconds, it was deemed completely unconscious. This procedure mimicked anesthesia administration during the scanning process and allowed us to verify the animal's vigilance level at each anesthetic dose administered during fMRI data acquisition.

We demonstrated that when 1.5% isoflurane was administered via a nosecone, 81% of animals were unable to correct from supine to prone position (Figure 2-3). This percentage was significantly higher than when lower doses of isoflurane were administered (no rats lost righting reflex at lower doses, Figure 2-3, Chi-square test, \( x^2=22.82, p<2\times10^{-6} \)). Therefore, 1.5% was the dose that induced LOC when isoflurane was administered via nosecone. It should be noted that the actual concentration of isoflurane inside the animal was lower than the concentration delivered due to the air dilution around the nose cone.

![Figure 2-3: Loss of Righting Reflex in Rats during AIU paradigm. The percentage of animals that were unable to correct from a supine to a prone position.](image)
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%, indicating that the majority of animals lost consciousness at the isoflurane concentration of 1.5% (delivered through a nosecone).

Data Processing & Analyses

The first 10 volumes of each rsfMRI run were discarded to allow magnetization to reach steady state. The data was preprocessed with conventional procedures as previously described (Liang et al., 2014a, 2015a; 2015b), including: registration to a segmented rat brain atlas, motion correction with SPM12, spatial smoothing (FWHM=1mm), regression of nuisance signals, as well as band-pass filtering (0.0085-0.1Hz). 16 nuisance signals were regressed out, including the signals from whiter 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 in order to avoid any spurious anticorrelations that may arise (Murphy et al., 2009).

Data were separately analyzed on a region-of-interest (ROI) and 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) (see Appendix). We derived FC by correlating the BOLD signal 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 absolute FC change was determined by subtracting the FC strength at the awake state from the corresponding FC strength at each anesthetized condition. This absolute FC change at the dose was then divided by the corresponding FC strength at the awake state (referred to as normalized FC change). To examine the change of information exchange for a local brain region (a voxel or ROI), we averaged the normalized FC change between that voxel (or ROI) and all other brain voxels (or ROIs) at each anesthetized condition (Olde Dubbelink, 2013). This averaged normalized FC change was used as the quantity to measure information exchange at the voxel/ROI.

To test our hypothesis that spontaneous neuronal activity becomes more random during AIU as a result of meaningful information processing loss, 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 white noise using a normal distribution and smoothed the white noise using the same temporal filter used in data preprocessing. All data
analyses were conducted using Mathematica software (Wolfram, Champaign, IL, USA.)
Chapter 3

AIU has a Global Impact on the Brain

Numerous literature studies have reported that AIU is associated with a disruption of FC within the thalamocortical and frontoparietal networks (Boveroux et al. 2010; Martuzzi et al., 2010; Deshpande et al., 2010; Vincent et al., 2007; Moller et al., 2009; Wang et al., 2011; Liang et al., 2013, Hudetz et al., 2015). To replicate these findings, we examined the absolute FC changes in connections between regions of interest (ROIs) in the thalamus, primary sensory-motor, parietal and prefrontal cortices (see Table 1-1 for the list of ROIs) between the awake state and an unconscious state (3% isoflurane) using the conventional subtraction method. The ROI definitions were based on Swanson Atlas (Swanson, 2004).

Figure 3-1 shows connections with significantly decreased (blue lines) and increased (red lines) FC within the thalamocortical and frontoparietal networks, displayed in the axial and coronal views (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.
Figure 3-1: **Significant changes in FC within regions of thalamocortical and frontoparietal networks at 3.0% isoflurane.** Horizontal and coronal views of the rat brain display significantly decreased (blue lines) and increased (red lines) FC between ROIs (listed in Table S1) in the thalamus, primary sensory cortex, parietal cortex, and prefrontal cortex (color-coded) from the awake state to 3.0% isoflurane (t-test, p < 0.05, False Discovery Rate corrected). P=posterior, A=anterior, R=right and L=left.

**Table 1-1: Brain regions in thalamus, primary sensory, parietal, and prefrontal cortices.**

<table>
<thead>
<tr>
<th>Thalamus</th>
<th>Primary sensory cortex</th>
<th>Parietal cortex</th>
<th>Prefrontal cortex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior nuclei, dorsal thalamus</td>
<td>Auditory areas</td>
<td>Ectorhinal area</td>
<td>Agranular insular area</td>
</tr>
<tr>
<td>Lateral geniculate complex</td>
<td>Gustatory area</td>
<td>Parietal region</td>
<td>Anterior cingulate area</td>
</tr>
<tr>
<td>Lateral nuclei, dorsal thalamus</td>
<td>Primary somatomotor area</td>
<td>Retrosplenial area</td>
<td>Infrafimbic area</td>
</tr>
<tr>
<td>Medial geniculate complex</td>
<td>Primary somatosensory area</td>
<td>Supplemntal somatosensory area</td>
<td>Orbital area</td>
</tr>
<tr>
<td>Medial nuclei, dorsal thalamus</td>
<td>Secondary somatomotor area</td>
<td>Ventral temporal association areas</td>
<td>Prelimbic area</td>
</tr>
<tr>
<td>Midline group, dorsal thalamus</td>
<td>Visceral area</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reticular nucleus thalamus</td>
<td>Visual areas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventral nuclei, dorsal thalamus</td>
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</tr>
</tbody>
</table>
We further examined anesthetic-induced FC changes across the whole brain. The brain was parcellated into 134 unilateral ROIs (Appendix). For each dose, we calculated the absolute FC change (i.e. ΔFC) between every two ROIs relative to the awake state (134 unilateral ROIs and 8911 connections in total). Intriguingly, this ΔFC highly depended on the corresponding FC strength at the awake state across all connections in the brain (Figure 3-2 A, r= -0.41, -0.49, -0.62, -0.60, -0.81, for isoflurane concentrations: 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 ΔFC 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, Figure 3-3 A). Furthermore, this dependency existed regardless of the spatial scale as evidenced by the consistency of the results obtained for all connections between every pair of brain voxels (Figure 3-2 B, ~6000 voxels and ~18 million connections, r= -0.60, -0.64, -0.71, -0.72 and -0.79 for isoflurane concentrations: 0.5%, 1.0%, 1.5%, 2.0% and 3.0%, respectively, p<10^{-200} for all concentrations). The difference in these correlations was statistically significant across doses for the voxel-wise analysis (one-way ANOVA, F(4,120) = 3.32, p = 0.01, Figure 3-3 B).
To investigate the information exchange at a local brain region during AIU, we first normalized the absolute FC change of each connection (i.e. ΔFC) at each anesthetized condition to the corresponding FC strength at the awake state.

Figure 3-2: **The absolute FC changes are dependent on the FC strength at the awake state.** Scatter plots of ROI- (A) and voxel-based (B) analyses show that the dependency between the absolute FC changes and the FC strength at the awake state becomes stronger at higher doses of isoflurane.

Figure 3-3: **Correlation (r) between the absolute FC changes and the FC strength at the awake state as a function of isoflurane concentration in both ROI- (A) and voxel-wise (B) analyses.** The difference in these correlations was 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.
(referred to as \textit{normalized FC change} hereafter). For each brain voxel (or ROI), we then averaged its \textit{normalized FC changes} with all other brain voxels (or ROIs), and this quantity was used as a measure of information exchange for this voxel (or ROI) (Olde Dubbelink, 2013). Figure 3-4 shows voxel-wise histograms (A) and spatial distributions (B) of the averaged \textit{normalized FC change} for all isoflurane doses. Strikingly, all voxels exhibited negative \textit{normalized FC change} for all doses, indicating an exclusive reduction of information exchange under anesthesia. In addition, the averaged \textit{normalized FC change} became spatially homogeneous at unconscious states (i.e. isoflurane dose $\geq 1.5\%$). These results collectively suggest a spatially uniform information exchange loss that widely extends across the whole brain during AIU.

\textbf{A.} \\
\textbf{B.}

\textbf{Figure 3-4:} The distributions and voxel-wise maps of the average \textit{normalized FC changes at each dose of isoflurane}. (A) Histograms show a dramatic reduction of the normalized FC at higher doses of isoflurane. (B) Spatial maps reveal a global, uniform reduction in normalized FC during unconsciousness.
Figure 3-4 A also demonstrated that, relative to the awake state, the overall FC strength across the brain was reduced by ~80% (i.e. normalized FC change < -0.8) after the animal lost consciousness (i.e. isoflurane dose ≥ 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, 2015). To test this hypothesis, we calculated Shannon entropy and mutual information of the BOLD signal at each condition. Here, entropy, which provides a measure of randomness in the BOLD signal, characterizes the amount of information that the signal contains, and mutual information describes the amount of information that is exchanged.

Figure 3-5 A 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 approached 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 unconsciousness deepened. This change in mutual information was also statistically significant across doses (Figure 3-5 B, 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.

![Graph of entropy and mutual information](image)

**Figure 3-5**: Entropy and mutual information at each isoflurane dose demonstrate that meaningful information is greatly diminished during isoflurane-induced unconsciousness. (A) The entropy of the BOLD signal increased to a value corresponding to random noise (~2.0), and (B) mutual information decreased as anesthetic depth increased. Bars: SEM.

The relatively uniform reduction of FC across the whole brain during unconsciousness appears to contradict a list of neuroimaging studies that highlight specific brain regions and circuits that are particularly vulnerable to anesthesia (e.g. thalamocortical and frontoparietal networks) (Whie & Alkire, 2003; Angel, 1991; Velly, 2007; Lee et al., 2009; Breshears et al., 2010).

However, this contradiction is most likely attributed to the difference in data analysis methods. Previous neuroimaging studies have relied on the subtraction method that statistically compares absolute FC changes between the awake and anesthetized states (Liang, King, Zhang, 2012; Barttfeld et al., 2015) or between different anesthetic depths (Vincent et al., 2007; Zhao et al., 2008; Liu et al., 2011; Hutchinson et al., 2014; Williams et al., 2010). As a result, these studies tend to highlight regions/networks that exhibit large changes in absolute FC.
Indeed, by using the conventional subtraction method our data showed significantly reduced FC in the thalamocortical and frontoparietal networks (Fig. 3-1). We also found that, in general, connections with stronger FC in the awake state tended to exhibit larger absolute FC changes during AIU (Fig. 3-2). Therefore, to reveal more subtle FC changes and also to account for the influence of the FC strength in the awake state on FC changes observed during AIU, we normalized the absolute FC changes 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 the extent to which the conventional subtraction method would reveal apparently different results, we identified connections with significant absolute FC changes (red dots in Figure 3-6, p < 0.005, uncorrected). It is clear from Figure 3-6 that with the use of the subtraction method, a specific set of connections were highlighted, while the global-scale proportional relationship revealed in the present study would no longer be as pronounced.
Figure 3-6: Scatter plots illustrating the relationship between the absolute FC changes (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 exhibited significant absolute FC changes during subtraction method analysis (t-test, p<0.005).
Chapter 4

Agent Invariance of AIU

To examine whether or not the global pattern of information exchange disruption during isoflurane-induced unconsciousness is agent-dependent, and also to rule out the possibility that the changes we observed result 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 (Nelson et al., 2003; Angel, 1991; 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 minutes after the bolus injection to maintain sedation (Nelson et al., 2003; Angel, 1991; 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 described above.

Similar to isoflurane-induced unconsciousness, we observed a strong dependency between the absolute FC changes at the dexmedetomidine-induced unconscious state and the FC strength at the awake state across all connections.
(Figure 4-1A, ROI-wise analysis, r = -0.74, p < 10^{-200}; Figure 4-1B, 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 normalized FC changes were drastically reduced by ~85% during dexmedetomidine-induced unconsciousness (Figure 4-1C), and the spatial distribution of this information exchange loss was homogenous across the brain during this unconscious state (Figure 4-1D). Moreover, like isoflurane-induced unconsciousness, we observed an increase in the entropy (Figure 4-1E) and a decrease in mutual information (Figure 4-1 F) during dexmedetomidine-induced unconsciousness. Collectively, these data suggest that the global disruption of information exchange is not agent specific, and may be an indication of an agent-invariant mechanism of AIU.
Our data provide evidence that the global reduction of information exchange during AIU is not dependent on the anesthetic agent used, as we show very consistent findings in rats anesthetized with dexmedetomidine—an anesthetic agent with a very different molecular mechanism from isoflurane.

Figure 4-1: **Dexmedetomidine and isoflurane-induced unconsciousness are characterized by the same patterns of information exchange loss.** (A and B) The absolute FC changes at the anesthetized state were dependent on the FC strength at the awake state for both ROI- (A) and voxel-based (B) analyses. (C and D) The distribution (C) and voxel-wise maps (D) of the averaged normalized FC changes during dexmedetomidine-induced unconsciousness. (E and F) The entropy (E) and mutual information (F) of the BOLD signal at the awake state and during dexmedetomidine-induced unconsciousness. Bars: SEM.
Isoflurane is a halogenated ether that affects both neural (Angel, 1993; Masamoto et al., 2007; Ries & Puil, 1999; Vahle-Hinz et al., 2007) and vascular (Ori et al. 1986, Lenz et al. 1998, Maekawa et al., 1986; Alkire et al.,1997) substrates, whereas dexmedetomidine is an alpha-2-adrenoceptor agonist with virtually no vascular effect and whose molecular action closely resembles that of natural sleep (Nelson et al., 2003). By performing the same experiment during dexmedetomidine-induced unconsciousness, we show that the global pattern of information exchange loss induced by isoflurane was not specific to the agent. This experiment also ruled out the possibility that the FC changes we observed were caused by the vascular effects of isoflurane (Lenz et al., 1998) considering that isoflurane reduces arterial blood pressure and increases cerebral blood flow (Lenz et al., 1998). Similar patterns of information exchange loss during dexmedetomidine- and isoflurane-induced unconsciousness suggest that this mechanism may be common across various anesthetic agents, or even, a general mechanism of unconsciousness. Future studies can extend this analysis to more anesthetic agents with distinct pharmacological profiles and to alternative forms of unconsciousness, such as sleep or coma.
Chapter 5

Advantages and Limitations

One major advantage of the current study is the utilization of the awake animal imaging paradigm established in our lab (Liang, King, Zhang, 2012; Liang, King, Zhang, 2013; Liang et al., 2011; 2012; 2014; 2015; 2015). Animal fMRI experiments typically rely on anesthesia to immobilize animals, which confounds the effects of the anesthetic agents being studied (Angel, 1991; Detsch et al., 1999; Vahle-Hinz et al., 2002). Consequently, without the ‘ground truth’ of brain activity/connectivity at the awake state (i.e. awake rsfMRI data), 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 systems-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 to determine the whole-brain FC changes during various unconscious conditions.

One potential limitation in the present study is the higher motion level in the rsfMRI data collected 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 across different levels of consciousness cannot explain the relatively uniform FC reduction that we observed during AIU. First, it is unlikely that larger motion at the awake state can lead to uniformly higher FC strength. In fact, motion tends to
reduce the FC strength in rsfMRI experiments (Van Dijk et al., 2012). Additionally, 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 those at anesthetized states (one-way ANOVA: F(4,36) = 2.08, p=0.09). Figure 5-1 shows that the pattern of information exchange loss obtained from this subset of data is almost identical to that from the full dataset.

Figure 5-1: ROI (A) and voxel-based (B) 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 were comparable (one-way ANOVA: F(4,36)=2.08, p=0.09). Similar results obtained in the subset suggest that our findings are not attributed to differences in the motion level across different consciousness levels.
Conclusion

Overall, the present thesis provides compelling neuroimaging evidence showing a global reduction of FC during AIU, which may reflect a 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 anesthetic agents with drastically different molecular actions, we suspect that this mechanism may be agent invariant. These findings allude to a common systems-level mechanism of AIU in which the information exchange capacity is affected in all brain regions/networks to a similar degree.

The uniform reduction of FC associated with anesthetized states might serve as a biomarker for unconsciousness that can be utilized in a clinical setting. Such a biomarker would effectively reduce anesthesia-related complications during surgical procedures by ensuring that the correct dosage for the desired anesthetic depth is administered. 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 (Laureys & Schiff, 2012).
Appendix

Rat Brain Parcellation

The rat brain was parceled into 134 unilateral regions of interest (ROIs) based in the anatomical partitions in Swanson atlas (Swanson, 2004). White matter and ventricle regions were excluded.
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