Diversity of Functional Connectivity Patterns is Reduced in Propofol-Induced Unconsciousness

Heonsoo Lee ^{1,*} Gyu-Jeong Noh,^{2,3} Pangyu Joo,¹ Byung-Moon Choi,³
Brian Henry Silverstein,⁴ Minkyung Kim,¹ Jisung Wang,¹
Woo-Sung Jung,^{1,5,6} and Seunghwan Kim¹

¹Department of Physics, Pohang University of Science and Technology, Pohang, Korea

²Department of Clinical Pharmacology and Therapeutics, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

³Department of Anesthesiology and Pain Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

⁴Translational Neuroscience Program, Wayne State University, Detroit, Michigan

⁵Department of Industrial and Management Engineering, Pohang University of Science and Technology, Pohang, Korea

⁶Asia Pacific Center for Theoretical Physics, Pohang, Korea

Abstract: *Introduction:* Recent evidence suggests that the conscious brain is characterized by a diverse repertoire of functional connectivity patterns while the anesthetized brain shows stereotyped activity. However, classical time-averaged methods of connectivity dismiss dynamic and temporal characteristics of functional configurations. Here we demonstrate a new approach which characterizes time-varying patterns of functional connectivity at the subsecond time scale. *Methods:* We introduce phase-lag entropy (PLE), a measure of the diversity of temporal patterns in the phase relationships between two signals. The proposed measure was applied to multichannel electroencephalogram (EEG), which were recorded from two distinct experimental settings: (1) propofol was administered at a constant infusion rate for 60 min ($n = 96$); (2) administration of propofol by a target effect-site concentration-controlled infusion with simultaneous assessment of the level of consciousness ($n = 10$). *Results:* From the first dataset, two substantial changes of the phase relationship during anesthesia was found: (1) the dynamics of the phase relationship between frontal channels became progressively less diverse and more stereotyped during unconsciousness, quantified as a reduction in PLE; and (2) the reduction in PLE was consistent across subjects. Furthermore, PLE provided better performance in the classification of states of consciousness than did phase-lag index, a classical time-averaged connectivity method. From the second dataset, PLE showed the highest agreement with the level of consciousness, compared to existing anesthetic depth indicators. *Conclusions:* This study suggests that a scarcity of functional configurations is closely associated with anesthetically induced unconsciousness, and shows promise as a basis for a new consciousness monitoring system during general anesthesia. *Hum Brain Mapp* 38:4980–4995, 2017. © 2017 Wiley Periodicals, Inc.

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Heonsoo Lee, Department of Physics, Pohang University of Science and Technology, Pohang, 37673, Korea

*Correspondence to: Heonsoo Lee; Department of Physics, Pohang University of Science and Technology: 77 Cheongam-Ro, Nam-gu, Pohang 37673, Korea. E-mail: leecj@gmail.com

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INTRODUCTION

Neural communication is inherently transient and non-stationary [Friston, 2000; Varela et al., 2001]. Synchrony emerges and disappears on a subsecond time scale; while it can be evoked by external stimuli [Lachaux et al., 1999; Massimini et al., 2005; Rodriguez et al., 1999], it can also arise spontaneously. Fluctuating functional connectivity patterns have been observed in the resting state, both in electroencephalography (EEG) [Stam and de Bruin, 2004; Thatcher et al., 2009; Yang et al., 2012] and in functional magnetic resonance imaging (fMRI) data [Allen et al., 2014; Hutchison et al., 2013].

Meanwhile, evidence from recent studies of consciousness suggest that state of consciousness is more closely associated with the temporal dynamics of functional network configuration, rather than the strength of static connectivity [Barttfeld et al., 2014; Casali et al., 2013; Hudetz et al., 2015, 2016; Sarasso et al., 2015]. Theories of consciousness predict that the waking state will be characterized by a rich repertoire of functional patterns while the unconscious brain will show lack of patterns [Dehaene and Changeux, 2011; Oizumi et al., 2014]. In animal fMRI studies, diverse functional connectivity patterns were observed during consciousness, while loss of consciousness due to anesthesia was associated with scarcity of patterns [Barttfeld et al., 2014; Hudetz et al., 2015].

However, current techniques for measuring connectivity in neurophysiological signals do not adequately account for the temporal dynamics in synchronization patterns. Popular phase synchronization methods, which use coherency or phase locking to infer connectivity [Mormann et al., 2000; Nolte et al., 2004; Stam et al., 2007], assume stationarity throughout the measurement period. The phase synchronization value is obtained by averaging phase differences across periods of several seconds, thereby disregarding the temporal dynamics within the window.

In this study we introduce a new measure, phase-lag entropy (PLE) that calculates diversity of the temporal patterns of the phase relationship. In contrast to the classical methods of phase synchronization, in which the strength of connectivity is of interest, the proposed measure reflects whether a given interaction between two signals consists of diverse or stereotypic connectivity patterns. In this way, PLE better reflects the time-varying dynamics of phase relationships, in which neural communication is embedded [Rodriguez et al., 1999; Varela et al., 2001]. We argue that the inclusion of temporal dynamics in the phase synchronization measure provides a more sensitive and

biologically relevant classification of connectivity states in neurophysiological signals.

Motivated by recent theories and investigations into consciousness, we applied the introduced measure, PLE, to EEG data recorded from healthy volunteers undergoing general anesthesia. We predicted that the diversity of connectivity patterns, quantified by PLE, would be a better predictor of the level of consciousness, than the strength of connectivity. Furthermore, the usage of stimulus-free resting state EEG, allowed us to investigate the feasibility of applying PLE in clinical practice for use in monitoring consciousness in anesthesia and brain-lesioned patients.

We measured PLE in two experimental EEG data sets. In the first study, 48 healthy volunteers received propofol at a constant rate over 60 min on two occasions; 3, 6, and 12 mg kg⁻¹ h⁻¹ for three groups (16 subjects per each group). The temporal behavior of the phase relationships among EEG signals and the corresponding PLE were investigated for each state of consciousness. When subjects were unconscious, the temporal patterns of phase relationships became stereotypic, which was captured well by a decrease in PLE.

In the second experiment, the ability of several EEG-based depth of anesthesia (DOA) indicators, including PLE, to predict the level of consciousness was investigated. This study was performed with administration of propofol by a target effect-site concentration-controlled infusion ($n = 10$) and simultaneous assessment of the level of consciousness using the Modified Observer's Assessment of Alertness/Sedation (MOAA/S) [Chernik et al., 1990]. Among the DOAs, PLE showed the strongest agreement with the MOAA/S suggesting potential clinical application for monitoring consciousness.

METHODS

Phase Coherence

Phase coherence (PC) measures phase locking between two signals [Mormann et al., 2000]. Consistent phase differences over time results in a high value of PC, which indicates the existence of synchrony between the two signals. For two independent signals, the phase differences will be equally distributed in $[-\pi, \pi]$, and the corresponding PC will be low.

To calculate PC, first, the instantaneous phase time series of each signal is obtained via a Hilbert transform. Then, the phase difference ($\Delta\phi_t$) can be calculated for each time point, t . The variability of the phase difference over time can be estimated as,

$$PC = \frac{1}{N} \left| \sum_{t=1}^N e^{i\Delta\phi_t} \right|,$$

where N is the number of time points within a given epoch. In practice, the epoch is chosen with a length of several seconds, and assumes stationarity of the data. PC has been widely used as a measure of phase synchrony and applied in functional connectivity analyses.

Phase-Lag Index

Phase-lag index (PLI) has been widely used for measuring phase synchrony in EEG and magnetoencephalogram data because it is less influenced by volume conduction and the choice of reference than other functional connectivity measures [Stam et al., 2007]. The basic idea behind PLI is that the presence of consistent nonzero phase-lags between two signals cannot be caused by the volume conduction effect, and instead represents genuine interaction. To estimate PLI, the instantaneous phase time series of brain signals are calculated using the Hilbert transformation, as in PC, and estimated as,

$$PLI = \frac{1}{N} \left| \sum_{t=1}^N \text{sign}(\Delta\phi_t) \right|,$$

where the sign ($\cdot$) function yields 1 if $\Delta\phi_t > 0$, and -1 if $\Delta\phi_t < 0$. Again, windows are chosen on the order of seconds, and stationarity is assumed. Independent signals or severe volume conduction yield $PLI \approx 0$; complete locking with nonzero phase-lag yields $PLI = 1$. Therefore, a high PLI value implies the existence of phase-lagged synchrony, and $PLI \approx 0$ can indicate either zero phase-lag synchrony, volume conduction, or independency.

Phase-Lag Entropy

In PC and PLI analyses, stationarity is assumed and phase locking is estimated within a given epoch, usually calculated on the order of seconds. However, the time scale for synchronization is in actuality much shorter. Synchrony emerges and disappears in tens or hundreds of milliseconds [Friston, 2000; Rodriguez et al., 1999; Stam and de Bruin, 2004; Varela et al., 2001]. Hence, PC and PLI cannot capture the temporal characteristics of phase relationships at the subsecond time scale. Still, they can show dynamic changes in synchrony level when averaged across trials which are time-locked to a certain event (e.g., task or stimulus) [Lachaux et al., 1999]. However, event-related paradigms are beyond the scope of this study. Here, we will focus on task- or stimulus-free time series data in which time-averaging technique is required.

The proposed measure, phase-lag entropy (PLE), incorporates the temporal dynamics of the instantaneous phase time series into the phase synchronization analysis. The

critical difference between PLE and the classical phase synchronization measure (PC and PLI) is that the former extracts temporal pattern of the phase relationship which spanning tens of milliseconds, while the latter just calculates averaged locking of phases without patterning process. Using this information, PLE is able to detect temporal changes in neural communication which cannot be observed using current phase synchronization methods. Specifically, PLE quantifies the diversity of temporal patterns of the phase relationship between two signals.

To calculate PLE, the phase difference is first symbolized in a binary fashion similar to the calculation of PLI; the symbol $s_t=1$ if $\Delta\phi_t > 0$ (first signal is phase leading the second signal), and $s_t=0$ if $\Delta\phi_t < 0$ (first signal is phase-lagging the second signal). Then, the vector S_t representing the temporal pattern of the phase relationship is given by

$$S_t = \{s_t, s_{t+\tau}, \dots, s_{t+(m-1)\tau}\}, t = 1, 2, \dots, N-(m-1)\tau,$$

where m and τ represent pattern size (word length) and time lag, respectively. For instance, with $m=3$, eight patterns ("000," "001," "010," "100," "011," "101," "110," and "111") can be generated. Finally, PLE is calculated by applying the standard Shannon entropy formula to the distribution of the phase patterns,

$$PLE = -\frac{1}{\log(2^m)} \sum_j p_j \log p_j,$$

where $0 \leq p_j \leq 1$ is the probability of the j^{th} pattern, as estimated by counting the number of times each pattern occurs in a given epoch. The normalization term in the denominator scales PLE to the range [0 1]. If a few patterns are dominant over other possible patterns, $PLE \approx 0$; if all patterns are equally probable, $PLE \approx 1$.

A scarcity in pattern diversity (i.e., $PLE \approx 0$) may imply stereotypic communication between two brain regions. For instance, if the first brain region is strongly affecting the second region with a non-negligible time delay, the pattern "111" would be dominant over the other seven patterns, and the corresponding PLE would be low. On the other hand, if two brain regions are interacting back and forth in complex fashion over time, the distribution of patterns will be close to uniform, resulting in high PLE.

Notice that in contrast to PC and PLI, PLE does not reflect whether two signals are correlated or independent, but indicates whether the given connection comprises many or few unique temporal patterns. In this way, PLE measures the diversity of connectivity patterns, as opposed to the strength of connectivity.

Figure 1 illustrates the fundamental examples of how PLE works in comparison with PLI. Three examples of simulated phase difference sequences are shown in Figure 1A,C,E. In each sequence, the number of positive phase differences and the number of negative phase differences are the same; that is, there are 50 data points of $s_t=1$ and 50 data points of $s_t=0$. However, the temporal dynamics

Figure 1.

PLE quantifies diversity of phase-lag patterns. Three examples of symbol sequence of phase difference are illustrated with corresponding phase-lag distribution and phase-lag pattern distribution. **(A)** symbol sequence, $s_t=1$ for the first half, and $s_t=0$ for the last half of data; this means $\Delta\phi > 0$ in the first half and $\Delta\phi < 0$ in the last half. **(B)** For the symbol sequence in **(A)**, the histogram of phase-lags shows equal numbers of 0 and 1, resulting in $PLI=0$ (left panel). The histogram of the phase-lag patterns shows two dominant patterns (000 and 111), resulting in $PLE=0.381$ (right panel). **(C)** s_t is repeatedly and regularly changing over time. **(D)** The histogram of phase-lags corresponding to

of s_t are different for the three examples. In Figure 1A, $s_t=1$ in the first half of the sequence and $s_t=0$ in the second half of the sequence. In Figure 1C, s_t is repeatedly and regularly changing with each time step. In Figure 1E, s_t is randomly distributed over time. The first two examples represent a phase relationship with regular dynamics, while the third example represents an irregular and disordered phase relationship. The histograms of phase-lags (left panels in Fig. 1B,D,E) clearly show completely symmetric distributions in all three cases, resulting in $PLI=0$. On the other hand, the histograms of phase-lag patterns are distinct (right panels in Fig. 1B,D,E). In the first and second examples, two dominant patterns exist; “000” and “111” for the first example, “101” and “010” for the second example ($m=3$ and $\tau=1$ were chosen). In the third example, all 8 patterns are almost equally distributed. $PLE=0.381$, 0.333 , and 0.994 for the first, second, and third examples, respectively. By employing the temporal dynamics of the phase relationship, PLE is thereby able to distinguish irregular from regular phase-lag dynamics.

Choice of two parameters—pattern size, m , and time lag, τ —may influence the value of PLE. If τ is too small, the

(C) shows equal numbers of 0 and 1, resulting in $PLI=0$ (left panel). In the histogram of phase-lag patterns, only two patterns (010 and 101) exist (right panel); this results in $PLE=0.333$. **(E)** s_t is randomly distributed over time. **(F)** The histogram of phase-lags shows equal numbers of 0 and 1, resulting in $PLI=0$ (left panel). The histogram of phase-lag patterns shows a relatively flat distribution over all 8 patterns; in this case, $PLE=0.994$. PLI cannot distinguish between these three examples because it only considers the distribution of phase-lags, ignoring the temporal information. On the other hand, PLE can distinguish (E) from (A) and (C), because it considers the temporal pattern of the phase-lags.

successive elements of the pattern s_t are strongly correlated; if τ is too large, the successive elements of the pattern s_t are almost independent. If m is too large, the distribution of phase-lag patterns becomes sparse, yielding a less precise entropy estimate. To choose appropriate τ , mutual information was calculated [Fraser and Swinney, 1986]. Group-averaged mutual information between two sequences of s_t drawn from experimental EEG data is shown in Supporting Information, Figure 1. The time delay factor at which mutual information decays to $1/e$ was 2 (~ 8 ms) and the minimum occurred at 11 (~ 43 ms). In this study, we chose an intermediate value, $\tau=6$ (~ 23 ms) [Kantz and Schreiber, 2004]. For the pattern size, we chose $m=3$. A search of the parameter space from $m=3\sim 6$ and $\tau=1\sim 11$ reproduced the original results, suggesting that the algorithm is robust to the choice of m or τ (Supporting Information, Fig. 3).

Relative Beta Ratio, Approximate Entropy, and Permutation Entropy

In the second study, we investigate the degree to which PLE and existing DOA indicators are correlated with

Figure 2.

Distinct spectral changes across different stages of anesthesia. **(A)** Group-median spectrogram before, during, and after propofol administration. Propofol was administrated with infusion rate (IR) = 3 (left panel, $n = 30$), 6 (middle panel, $n = 29$), or 12 (right panel, $n = 31$) $\text{mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$. The IR was constant for 60 min. Higher IR (e.g., $\text{IR} = 12 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) promotes deeper state of anesthesia. The white dotted vertical line at 0 min indicates the propofol infusion start and the second white dotted vertical line at 60 min indicates the end of propofol. **(B)** Time course of averaged $\alpha\beta$ power (8–30 Hz) and δ power (0.5–4 Hz). Curves are smoothed with moving average filter. Different

rates of propofol infusion induce distinct power changes. A biphasic effect of $\alpha\beta$ power and δ power is shown with $\text{IR} = 12 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ (right panel). Red and green arrows above each box indicate LOC and ROC points, respectively, for each subject. Vertical colored bars indicate time periods analyzed from each state: baseline consciousness (BC): black ($n = 88$); sedation (A1): magenta ($n = 30$); $\alpha\beta$ power peak (A2): red ($n = 58$); slow oscillations (A3): yellow ($n = 31$); recovery of consciousness (RC): green ($n = 69$). See Methods section for state-identification criteria. [Color figure can be viewed at wileyonlinelibrary.com]

consciousness, as indexed by the MOAA/S. Four DOA indicators, Bispectral index (BIS; Aspect Medical System, Newton, MA), relative beta ratio (RBR), approximate entropy (ApEn), and permutation entropy (PeEn) were used in the analysis. The BIS values were obtained during the experiment from the BIS monitor while PLE and other DOA indicators were obtained via offline calculation.

The RBR is a subparameter of the BIS algorithm which quantifies spectral changes in anesthesia. The RBR is defined as a ratio of γ -power (30–47 Hz) and a frequency band composed of high α - and low β -power ($\alpha\beta$; 11–20 Hz):

$$\text{RBR} = \log \frac{\text{Power (30–47 Hz)}}{\text{Power (11–20 Hz)}}.$$

Because $\alpha\beta$ power has a biphasic pattern, that is, during anesthetic induction, an initial increase of $\alpha\beta$ power is followed by a decrease at higher anesthetic concentration (Fig. 2), the RBR is also expected to exhibit biphasic effect. In particular, the RBR has concordant relationship with the level of alertness/sedation such that in the BIS algorithm it is mostly used during sedation and light anesthesia.

The ApEn measures the predictability of subsequent time series values, based on the knowledge of the equidistant past values of time series [Pincus, 1991]. A high ApEn value indicates random or unpredictable dynamics while a low ApEn value implies regular or deterministic dynamics. To calculate the ApEn, an embedded time series is first obtained such that,

$$X_t = \{x_t, x_{t+\tau}, \dots, x_{t+(m-1)\tau}\}, \quad t = 1, 2, \dots, N-(m-1)\tau,$$

where x_t is EEG amplitude at time t , m is embedding dimension, and τ is the time lag; $\tau=1$ for ApEn calculation [Bruhn et al., 2000a; Pincus, 1991]. Second, the correlation sum is calculated from the embedded time series,

$$C_i^m(r) = \frac{1}{N-m-1} \sum_{j=1}^{N-m-1} \Theta(r - \|X_i, X_j\|).$$

where $\Theta(\cdot)$ denotes a Heaviside step function and $\|\cdot\|$ implies maximal distance between two vectors. The distance criteria r was fixed to be 0.2 standard deviation of

amplitudes within each epoch as in previous literature [Bruhn et al., 2000a]. Finally, the ApEn is defined as,

$$\text{ApEn}(m, r, N) = \frac{1}{N-m-1} \sum_{i=1}^{N-m} \log C_i^m(r) - \frac{1}{N-m} \sum_{i=1}^{N-m} \log C_i^{m+1}(r).$$

Many studies reported that the ApEn is decreased during general anesthesia, suggesting anesthetic suppresses the complexity of neural dynamics [Bruhn et al., 2000a,b; Noh et al., 2006]. In our study, the parameter set that provides the most concordant relationship with the level of consciousness was selected; F4 for active channel, F7 for reference channel, $m=2$, and downsampled to 128 Hz (Supporting Information, Table III).

Permutation entropy (PeEn), one of the best DOA indicator, is estimated from single channel signal using a symbolic transformation with pattern length m and time lag τ [Bandt and Pompe, 2002; Olofsen et al., 2008]. The patterns are described by order relations between present and equidistant past amplitude values of time series. For an N -point time series, delay reconstruction is conducted as in ApEn. Rearranging X_t in ascending order yields $\{x_{t+(i_1-1)\tau} \leq x_{t+(i_2-1)\tau} \leq \dots \leq x_{t+(i_m-1)\tau}\}$, where i_k denotes associated index of the k^{th} rank. Using Shannon entropy formula, PeEn is defined as

$$\text{PeEn} = -\frac{1}{\log(m!)} \sum_j p_j \log p_j.$$

In this study, the parameter set that provides the most concordant relationship with the level of consciousness was selected; $m=3$ and $\tau=2$ (Supporting Information, Table IV).

Experiments and EEG Recordings

Experimental data sets consisted of two studies; both were approved by the Institutional Review Board of the Asan Medical Center (Seoul, Korea) and written informed consent was obtained from all participants. Exclusion criteria were allergy to propofol, history of central nervous system diseases, abnormal laboratory findings with clinical significance, or body weight that was not within 30% of ideal body weight.

In the first study, 48 healthy volunteers were divided into three groups (16 subjects on each). Each of the three groups received propofol at 3, 6, and 12 mg·kg⁻¹·h⁻¹ over 60 min. The different infusion rates (IR) induced distinct neurophysiological and behavioral responses; a lower IR induced light stage of anesthesia while a higher IR induced a deeper level of anesthesia. The experiments were conducted with an identical protocol on two separate days, 1 week apart. The same experimental protocols with same IRs were conducted on two occasions: for the first

trial, long-chain triglyceride (LCT) propofol (Diprivan[®], AstraZeneca, London, UK) was used, and for the second trial, microemulsion propofol (Aquafoi[™], Daewon Pharm. Co. Ltd., Seoul, Korea) was used. Regarding the two trial data sets, we assumed that the anesthetic effects across two trials were different at the 1 week interval even if the two EEG recordings were from the same subject. Thus we consider the two data sets of 48 subjects as independent from one another. There was no qualitative difference in the results between the two trials.

Loss of consciousness (LOC) was tested every 10 s after propofol administration by checking for loss of response to verbal command ("open your eyes"). If respiratory depression occurred, lungs were manually ventilated with 100% O₂ through a facemask to maintain an end-tidal CO₂ concentration $35 \leq [\text{CO}_2] \leq 45$ mm Hg. Manual ventilation was discontinued when the spontaneous respiratory rate exceeded 12 breaths/min and end-tidal [CO₂] was <45 mm Hg. Recovery of consciousness (ROC) was assessed every 10 s after the discontinuation of propofol infusion by eye opening to a verbal command. All volunteers were instructed to close their eyes during the study period. The EEG of 7 channels (Fp1, Fp2, F3, F4, Cz, P3, and P4) referenced to A2, 10–20 system) was recorded with a sampling frequency 256 Hz and 16-bit analog-to-digital precision using a QEEG8 (LXE3208, Laxtha Inc., Daejeon, Korea). The 7 EEG channels were chosen considering the importance of frontal synchronization and frontal-parietal connectivity in general anesthesia [Boly et al., 2012; Cimenser et al., 2011; Lee et al., 2013b; Supp et al., 2011]. After a 4 min baseline measurement, raw EEG was recorded continuously for up to 120 min after administration of propofol. The average age was 49.6 ± 17.7 years (range, 21–74 years).

Five data sets were lost after recording (2, 2, and 1 from IR= 3, 6, and 12 mg·kg⁻¹·h⁻¹, respectively) and two data sets from IR= 6 and 12 mg·kg⁻¹·h⁻¹ were excluded from the analysis due to severe noise contamination. Therefore, the total number of data sets used in the analysis was, $n=30, 29$, and 31 , for IR=3, 6, and 12 mg·kg⁻¹·h⁻¹ groups, respectively.

The second study included 10 patients aged 55.6 ± 14.8 years (31–72 years) who were scheduled for elective surgery. LCT propofol was administered by a target effect-site concentration-controlled infusion (Asan Pump, version 1.3, Bionet Co., Ltd., Seoul, Korea) using the modified Marsh model [Struys et al., 2000]. The level of consciousness was assessed using the MOAA/S scale [Chernik et al., 1990; Schmidt et al., 2007] every 30 s after administration of propofol (Supporting Information, Table I). At progressively deeper levels of anesthesia, subjects faded from a fully conscious state, through light sedation, into deep sedation (MOAA/S 5 to 0). In deep sedation (MOAA/S of 0), subjects show no response to painful stimuli (trapetius squeeze). The target effect-site concentration of propofol was started at 1.0 µg·mL⁻¹ and was increased by 0.2 µg·mL⁻¹ every 2 min until the MOAA/S

TABLE I. Behavioral and EEG features of five states

Stage	Response to verbal command	EEG features
BC (baseline consciousness)	Yes	γ activity/posterior α activity
A1 (light sedation)	Yes	β , γ activity
A2 (deep sedation)	No	Maximal α activity/reduced β , γ activity
A3 (general anesthesia)	No	Maximal δ activity/reduced β , γ activity
RC (return of consciousness)	Yes	β , γ activity

scale reached 0. EEG was recorded from 12 channels (Fp1, Fpz, Fp2, F3, F4, F7, F8, P3, P4, P5, P6, and Cz referenced to A2, 10–20 system) using a QEEG32 system (LXE3232-RF, Laxtha Inc., Daejeon, Korea). After a 3 min baseline measurement, EEG was recorded continuously for up to 3 min after the MOAA/S scale reached 0.

Preprocessing of EEG Data

Because different states of anesthesia exhibit distinct EEG characteristics, we divided the data into 5 states according to behavioral and neurophysiological features (Fig. 2 and Table I): (1) baseline consciousness (BC), defined as 96 s before the propofol infusion; (2) first stage of anesthesia, or sedation (A1), defined as 96 s before the end of propofol infusion in subjects with $IR = 3 \text{ mg kg}^{-1} \text{ h}^{-1}$. For subjects who fully lost consciousness, A1 is defined as 96 s before the LOC point; (3) second stage of anesthesia, or $\alpha\beta$ power peak (A2), defined as 96 s after the peak of 8–30 Hz power, for subjects with $IR = 6$ and $12 \text{ mg kg}^{-1} \text{ h}^{-1}$; (4) third stage of anesthesia, or slow oscillations (A3): defined as 96 s after the peak of δ power (0.5–4 Hz). δ power was calculated after $\alpha\beta$ power reduced to 70% of its maximum value. A3 is only selected for subjects with $IR = 12 \text{ mg kg}^{-1} \text{ h}^{-1}$; (5) return of consciousness (RC): defined as 96 s after the ROC point. Although some data sets with $IR = 12 \text{ mg kg}^{-1} \text{ h}^{-1}$ showed a burst-suppression pattern ($n = 12$), we did not analyze the burst-suppression period in this study. The PC, PLI, and PLE were calculated within 8 s epochs; thus, in total, 12 ($=96/8$) values are obtained per each state per each subject. Notice that subjects could respond to the verbal command in BC, A1, and RC and not in A2 and A3.

For the classification performance test, conscious and unconscious states were also defined in each subject. Data for the conscious state was defined as 4 min before the start of propofol; data for the unconscious state was defined as 4 min after the LOC point. Therefore, in total, 30 ($=240/8$) values of PLI and PLE were obtained per each state per each subject. Data sets without an LOC point were excluded in this analysis.

For the first study, EEG artifacts were visually inspected and epochs containing amplitude $>200 \text{ } \mu\text{V}$ were rejected. For the second substudy, only automatic inspection (rejection for amplitude $>200 \text{ } \mu\text{V}$) was applied. The RBR, ApEn,

and PLE were also calculated within 8 s epochs. For the BIS, the median of 8 s period was selected for comparison.

It is well known that propofol-induced rhythmic activity starts in the β frequency band and its central frequency progressively slows down to the α frequency regime [Cimenser et al., 2011; Purdon et al., 2013]. Therefore, we chose a relatively broad frequency band, 8–30 Hz ($\alpha\beta$ bands). Zero-phase bandpass filtering with an FIR filter of order 500 was conducted.

Statistical Analysis

For the PC, PLI, and PLE analyses in the first study, we applied Kruskal–Wallis test to compare the 5 states. Multiple comparisons were thresholded for significance using the Tukey–Kramer method to adjust the P value; P values <0.05 were considered to represent significant changes across the states ($*P < 0.05$; $**P < 0.01$; $***P < 0.001$).

To show that the decrease of PLE due to propofol does not merely reflect a slow-down of brain activity, we compared the original PLE results with values resulting from a surrogate data set (PLE_{surr}). For each epoch, 19 surrogate data were generated using the phase randomization method [Schreiber and Schmitz, 2000], and the same calculation of PLE was applied. With this procedure, the correlation between the two signals is completely disrupted while preserving the spectral characteristics of each signal. PLE_{surr} represents the median values of 19 PLEs from the 19 surrogate datasets.

We investigate the factors by which the effect of propofol on each measure (PC, PLI, PLE, or PLE_{surr}) exceeds interindividual baseline (before anesthesia) variability. The ratio of the effect of propofol to interindividual variability, $\sigma(w)$, is defined at each epoch, w , as the difference between individual values, $M_{k,w}$, and median individual baseline value, $\tilde{M}_{k,B}$, averaged over all subjects, divided by the SD of all individual median baseline values, $SD(\tilde{M}_{k,B})$, [Bruhn et al., 2002; Noh et al., 2006],

$$\sigma(w) = \frac{\frac{1}{n} \sum_{k=1}^n (M_{k,w} - \tilde{M}_{k,B})}{SD(\tilde{M}_{k,B})},$$

where B and k denote the baseline state and individual subject, respectively, n is the total number of subjects, and $(\tilde{\cdot})$ implies the median over all epochs in the baseline state. If a measure changes prominently compared to its baseline fluctuation, it will give rise to a high absolute value of σ .

Figure 3.

Phase-lag patterns become stereotypic from BC through A1 to A2. Distribution of phase-lags, phase-lag sequence, and distribution of phase-lag patterns across 5 states; first col: BC ($n = 88$); second col: A1 ($n = 30$); third col: A2 ($n = 58$); fourth col: A3 ($n = 31$); and fifth col: RC ($n = 69$). **(A)** The distribution of phase differences ($\Delta\phi$) between Fp2 and F4 channels. Data from all subjects are aggregated. From BC to SE, phases become less coherent. From A1 to A2, phases become biased toward approximately 340° ; this means that the Fp2 signal tends to be phase-lagged to the F4 signal. The distribution of phase differences is still biased during A3. In RC, the biased distribution returns to 0° , while coherence remains low as in SE. **(B)** Representative bandpass filtered EEG signals of the Fp2 and F4 channels (blue and red curves, respectively, in upper panels) and corresponding phase-lag sequences (lower panels). From BC

through A1 to A2, s_t persists longer in one value; that is, it does not change often. Also in A2, $s_t=0$ becomes dominant reflecting the biased distribution of phase difference in A2. In A3, s_t changes often than it does in A2, but still biased toward $s_t=0$. The phase-lag sequence of RC is similar to that of SE. **(C)** Probability distribution of phase-lag pattern. The patterns are sorted in descending order of the probability. From BC through A1 to A2, one dominant pattern (000) emerges and other patterns diminish such that the distribution becomes skewed; this clearly comes from the biased distribution of $\Delta\phi$ and its persistent duration over time. This change will decrease entropy from BC through A1 to A2. The distribution then becomes flat from A3 to RC, becoming similar to SE. Error bars indicate standard deviation over subjects. [Color figure can be viewed at wileyonlinelibrary.com]

We also compared the performance of PLI and PLE in the classification of conscious state. A receiver operating characteristic curve was obtained for each subject ($n = 69$) after fitting a logistic regression model to the PLI or PLE, using the binary consciousness score (1 or 0). Performance was assessed as accuracy and as area under the curve (AUC).

In the analysis of the second study, prediction probability (P_K) was used to assess the correlation of each index with each subject's level of consciousness [Smith et al., 1996]. P_K quantifies the correlation between the observed level of consciousness (measured by, MOAA/S; [Struys et al., 2000]) and calculated indexes (BIS, RBR, ApEn, PeEn, and PLE) that have different unit scales. $P_K=1$ implies complete agreement between the observed level of consciousness and the calculated

index, $P_K=0.5$ represents a random relationship, and $P_K=0$ indicates complete disagreement. The standard error of P_K was computed using the jackknife method. All analyses were conducted in MATLAB (2015a, MathWorks, Natick, MA).

RESULTS

Phase Lead and Lag Relationship and Its Temporal Behavior During Anesthesia

Figure 3 demonstrates changes in the phase relationships between Fp2 and F4 across 5 states of anesthesia. In BC, the phase differences are relatively coherent and distributed around 0° (Fig. 3A). After anesthesia, the

coherence diminishes. Importantly, as the anesthetic deepens, the phase differences in A2 are biased toward approximately 340° , indicating that the Fp2 signal tends to be phase-lagged to the F4 signal. A similar feature is also observed in the A3 state. In RC, the phase differences are distributed near 0° again, but the coherence remains low as in A1. Note that subjects responded to the verbal command in the BC, A1, and RC states, but not in the A2 and A3 states. The symbol sequence (s_i) of the phase differences in BC has a complex temporal behavior (Fig. 3B), similar to the artificial random sequence in Figure 1E. From BC through A1 to A2, s_i tends to persist at a single value for longer periods; that is, the phase lead and lag relationship does not change often. Moreover, in the A2 state, due to the biased distribution of phase differences, $s_i = 0$ becomes dominant over $s_i = 1$. The probability distribution for each pattern is relatively flat in the BC state (Fig. 3C), indicating diverse functional connectivity patterns. After anesthesia, from BC, through A1, to A2, one dominant pattern ("000") emerges; this clearly results from the biased distribution of the phase differences and its prolonged duration in a single value. The distribution of the A3 state is similar to that of the A2 state. The distribution becomes relatively flat in RC, similar to that of A1. The phase lead and lag relationships and temporal behavior of other prefrontal-frontal pairs (Fp1-F3, Fp1-F4, and Fp2-F3) demonstrates very similar result to those seen in Fp2-F4.

Decrease of Phase-Lag Pattern Diversity During General Anesthesia

Next we measured PLE from all 21 channel pair combinations for comparison with PC and PLI. After anesthesia PC tends to decrease, but does not recover in RC state (Fig. 4A). In PLI, the most prominent change occurs during the transition from A1 to A2, mostly in prefrontal-frontal connections (Fig. 4B); this results from the biased distribution of phase differences in A2 (Fig. 3A). The increased prefrontal-frontal PLI diminishes in A3 and further dissolves in RC. PLE shows the most pronounced changes across the 5 states compared to PC and PLI (Fig. 4C). Overall, PLE is relatively higher during BC and is low during anesthesia, suggesting that anesthesia suppresses diverse functional connectivity patterns. In BC, PLE in prefrontal and frontal areas is higher than PLE in central and parietal areas. A large decrease is seen in the transition from BC to A1, across all channel pairs. In A2, PLE values decrease further and the changes are most pronounced in prefrontal-frontal connections; this results from the biased distribution of phase differences and its prolonged duration at the same value (Fig. 3A). PLE increases from A2 to A3 exhibiting biphasic effect, and further increases in RC.

Because the most salient changes across the 5 states appeared in prefrontal-frontal connections, the PC, PLI, and PLE values averaged over the corresponding 4 channel pairs (Fp1-F3, Fp1-F4, Fp2-F3, and Fp2-F4) were used for

statistical analysis and follow-up analyses (Fig. 4D). In PC, statistical significance was found in 5 out of 10 state pairs (BC-A1: $P < 10^{-4}$, BC-A2: $P < 0.01$, BC-A3: $P < 0.001$, BC-RC: $P < 10^{-8}$, and A2-RC: $P < 0.01$). In PLI, statistical significance was found in 6 state pairs (BC-A2: $P < 10^{-8}$, BC-A3: $P < 10^{-5}$, A1-A2: $P < 10^{-7}$, A1-A3: $P < 0.01$, A2-RC: $P < 10^{-8}$, and A3-RC: $P < 10^{-7}$). PLI during conscious states (BC, A1, and RC) was significantly lower than during unconscious states (A2 and A3), but progressive changes from BC, through A1, to A2 were not observed. In PLE, 8 out of 10 state pairs show statistical significance (BC-A1: $P < 0.001$, BC-A2: $P < 10^{-8}$, BC-A3: $P < 10^{-8}$, BC-RC: $P < 10^{-5}$, A1-A2: $P < 10^{-7}$, A1-A3: $P < 0.01$, A2-RC: $P < 10^{-8}$, and A3-RC: $P < 10^{-4}$). PLE during conscious states (BC, A1, and RC) was significantly higher than during unconscious states (A2 and A3). In addition, a progressive decrease from BC, through A1, to A2 was observed. PLE can also discriminate BC, a fully conscious state, and RC, a period right after the ROC point.

Figure 4E exhibits the time courses of σ for all 4 measures, PC, PLI, PLE, and PLE_{surr} . In all IR conditions, change in $\sigma(PLI)$ is the most salient among the 4 measures, indicating that the effect of propofol on PLE, relative to its interindividual baseline variability, is the highest. $|\sigma(PLI)| > |\sigma(PLI_{surr})|$ assures that the decrease of PLE due to propofol is not merely the reflection of a power spectrum change. With IR = 12 mg·kg⁻¹·h⁻¹, all measures exhibit biphasic effects (similar to $\alpha\beta$ power; Fig. 2).

Lack of Phase-Lag Pattern Diversity is Closely Associated With Loss of Consciousness

Next we compared the ability of PLE and PLI to classify conscious state on an individual subject level. Although the PLI in the unconscious state is higher than the PLI in the conscious state in the group-level analysis, some subjects show opposite results: 13 out of 69 subjects show lower PLI in the unconscious state. By contrast, individual level changes in PLE match the group level analysis; all 69 subjects exhibit lower PLE in the unconscious state versus the conscious state.

Classification performances of PLI and PLE were tested for each subject with a receiver operating characteristic curve (AUC) after fitting a logistic regression model. The AUC for PLE is significantly higher than that of PLI: AUC (PLI) = 0.799 ± 0.150 , AUC (PLE) = 0.963 ± 0.086 ($P < 0.001$, paired t test). Accuracy is also higher in PLE: accuracy (PLI) = 0.802 ± 0.112 , accuracy (PLE) = 0.954 ± 0.089 ($P < 0.001$, paired t test).

The qualitative changes in PLE near the transition points were also investigated. PLE before and after the LOC point show a substantial difference (Fig. 6). The IR = 12 mg·kg⁻¹·h⁻¹ condition promotes more abrupt changes across the LOC point than lower IR conditions; the PLE changes [−30s 30s] to LOC point were -0.007 ± 0.023 , -0.007 ± 0.029 , and -0.031 ± 0.032 , for IR = 3, 6, and 12,

Figure 4.

Progressive decrease in phase-lag pattern diversity is quantified by PLE. (A) Average PC matrix from 5 states: first col: BC ($n = 88$); second col: A1 ($n = 30$); third col: A2 ($n = 58$); fourth col: A3 ($n = 31$); fifth col: RC ($n = 69$). (B) Average PLI matrix from 5 states. PLIs between prefrontal and frontal channels are increased in A2 compared to BC and SE. From A2 to A3, prefrontal-frontal PLI decreases. (C) Average PLE matrix from 5 states. From BC through A1 to A2, PLE decreases in all EEG channel pairs. The change is most pronounced in prefrontal-frontal connections. From A2 through A3 to BS, PLE increases. (D) PC, PLI, and PLE from prefrontal-frontal channel pairs. The measures are averaged over 4 channel pairs (Fp1-F3, Fp1-F4, Fp2-F3, and Fp2-F4) and subjects. Compared to PC and PLI, PLE shows a progressive decrease from BC through A1 to A2 with statistical significance. The statistical significances between BC, SE, and A2 are emphasized with red colored stars. The error bars indicate standard deviation (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; adjusted P values after Tukey's multiple comparison test). (E) The effects of propofol on PC, PLI, and PLE are

assessed and compared over time. The curves represent the factors by which the average effect of each measure (PC, PLI, PLE, and PLE_{surr}) exceeds interindividual baseline variability (σ). Red arrows indicate LOC points and green arrows indicate ROC points for each subject. Curves are smoothed with moving average filter. PLE_{surr} is calculated after eliminating the connectivity between two signals (see Methods for details). When IR = 3 mg·kg⁻¹·h⁻¹ (upper panel, $n = 30$), PC, PLI, and PLE_{surr} barely change compared to their baseline values. PLE decreases monotonically during anesthesia. When IR = 6 mg·kg⁻¹·h⁻¹ (middle panel, $n = 29$), PLE shows higher ratio of changes with respect to interindividual baseline variability than PC, PLI, and PLE_{surr}. [$PLE > PLE_{surr}$] indicates that the scarcity of phase-lag patterns under anesthesia cannot be explained solely by spectral characteristics of each signal. When IR = 12 mg·kg⁻¹·h⁻¹ (lower panel, $n = 31$), PLI, PLE, and PLE_{surr} show biphasic effect as in power changes. In all 3 cases, the propofol effect on PLE is most salient among the 4 measures (see Supporting Information, Fig. 2 for time course of PeEn and ApEn).

respectively. However, the most abrupt increase across the ROC point is seen in the IR = 6 mg·kg⁻¹·h⁻¹ group; the PLE changes [-30s 30s] to ROC point were 0.017 ± 0.035 , 0.049 ± 0.043 , and 0.022 ± 0.040 , for IR = 3, 6, and 12, respectively. The recovery curve of IR = 12 mg·kg⁻¹·h⁻¹ group was relatively gradual.

Progressive Decrease of PLE During Progressive Loss of Consciousness

In the second study, we investigated the agreement of EEG-based DOA indicators with the level of consciousness as measured with the MOAA/S. Figure 7B exhibits

Figure 5.

Lack of phase-lag pattern diversity is associated with loss of consciousness. **(A)** Comparison of PLI (upper panel) and PLE (lower panel) in distinguishing states of consciousness. For each subject, median PLI and PLE over 30 data points per state (4 min in baseline and 4 min after LOC points, respectively) are obtained. PLI values do not consistently distinguish between conscious states across subjects; in contrast, PLE of unconscious state is lower than that of conscious state in all subjects ($n = 69$). **(B)**

Area under the curve (AUC) and accuracy from the receiver operating characteristic curve are obtained for each subject. PLE shows better classification performance both in AUC (lighter left bars) and accuracy (darker right bars). AUC of PLI = 0.799 ± 0.150 ; AUC of PLE = 0.963 ± 0.086 ; accuracy of PLI = 0.802 ± 0.112 ; accuracy of PLE = 0.954 ± 0.089 . Error bar is standard deviation. (***) $P < 0.001$; paired t test.

boxplots of five measures (BIS, RBR, ApEn, PeEn, and PLE) with corresponding MOAA/S scores. In PLE, the boxes for different MOAA/S scores overlap to a lesser degree, compared to the other four measures. The P_k of PLE is also the highest among the measures, suggesting that PLE has the most concordant relationship with the level of consciousness; $P_k = 0.834 \pm 0.012$, $P_k = 0.794 \pm 0.016$, $P_k = 0.887 \pm 0.010$, $P_k = 0.903 \pm 0.009$, and $P_k = 0.930 \pm 0.007$, for BIS, RBR, ApEn, PeEn, and PLE, respectively. For RBR, ApEn, PeEn, and PLE, the parameters (EEG channel, reference, and embedding parameters) which give the highest P_k were chosen (RBR: Fp1 for active channel, F8 for reference; ApEn: F4 for active channel, F7 for reference, $m=2$, and downsampled to 128 Hz; PeEn: F4 for active channel, F7 for reference, $m=3$, and $\tau=1$; PLE: Fp1, Fp2, F3, and F4 for active channels, A2 for reference, $m=3$, and $\tau=1$). Results with different parameters are shown in Supporting Information.

DISCUSSION

This study demonstrates that the diversity of functional configurations, quantified as PLE, rather than strength of functional connectivity, is associated with conscious state. The functional configuration among neurobiological signals was evaluated by extracting the temporal pattern of the phase relationships between the signals. The anesthesiologically induced unconscious state exhibited a substantial decrease of PLE, suggesting an impairment of flexible corticocortical communication. The ability of PLE to accurately classify conscious state, together with efficient and simple computation, suggests our method as a potential clinical index for monitoring consciousness during anesthesia.

Temporal Pattern of Phase Relationships in a Subsecond Time Scale

Almost all phase synchronization methods require stationarity of data with rather long times series ($\sim$ several seconds), to reliably estimate the strength of functional connectivity [Mormann et al., 2000; Nolte et al., 2004; Stam et al., 2007]. However, experimental studies suggest that flexible communication requires not only the creation but also the dissolution of synchronization within a subsecond time scale. For example, ERP studies show that neural activity waxes and wanes, and synchrony can appear and disappears within a fraction of a second [Lachaux et al., 1999; Rodriguez et al., 1999]. Also, in the resting state, rich and dynamic functional connectivity patterns arise from spontaneous brain activity [Stam and de Bruin, 2004; Thatcher et al., 2009; Yang et al., 2012]. Our study reveals that the conscious state consists of diverse patterns of functional connectivity whereas the anesthetized state has a few dominant patterns, suggesting stereotypic communication among brain areas. Unlike its static analog, PLI, in which the temporal dynamics of phase relationships are neglected, PLE can successfully evaluate the state and the level of consciousness during anesthesia. This implies that important temporal information can be lost when using average functional connectivity.

PLE Reinterprets Previously Reported Anesthetic-Induced Frontal Synchrony

We demonstrate two prominent features of phase relationships between prefrontal and frontal EEG signals during propofol-induced unconsciousness. First, the prefrontal signal is phase-lagged to the frontal signal such that the

Figure 6.

Qualitative changes of PLE near transition points. **(A)** Three representative subjects show distinct time courses of PLE. PLE is calculated from 4 channel pairs (Fp1-F3, Fp1-F4, Fp2-F3, and Fp2-F4) and averaged. Red line indicates LOC, whereas green line indicates ROC. Subject 28 (IR = 3 mg·kg⁻¹·h⁻¹) shows a continuous decrease in PLE near LOC and an abrupt increase near ROC. The increase of PLE is concurrent with the ROC point. PLE of subject 105 (IR = 6 mg·kg⁻¹·h⁻¹) exhibits a steeper decrease than that of Subject 28 near the LOC; after anesthesia cessation, the increase of PLE is also abrupt and concurrent with the ROC point. Subject 47 (IR = 12 mg·kg⁻¹·h⁻¹) shows a biphasic pattern similar to that seen in $\alpha\beta$ power (Fig. 2B). An abrupt decrease of PLE near LOC is shown; in contrast, recovery of PLE near ROC is relatively continuous, distinct from those of subject 28 and 105. **(B)** Group-averaged PLE matrix

between all channel pairs. Left most 4 panels are from IR = 3 mg·kg⁻¹·h⁻¹ ($n = 12$), middle 4 panels are from IR = 6 mg·kg⁻¹·h⁻¹ ($n = 26$), and right most 4 panels are from IR = 12 mg·kg⁻¹·h⁻¹ ($n = 31$), respectively. Each matrix represents PLEs of 4 min before LOC, 4 min after LOC, 4 min before ROC, and 4 min after ROC, from left to right. The broad loss of PLE after LOC (red to blue shift) and recovery of PLE after ROC (blue to red shift) can be seen. **(C)** Time course of prefrontal–frontal PLE near the transition points. PLE is averaged over all subjects. Error bars indicate standard error. Near the LOC point, PLE decreases faster for higher infusion rates; from left panel (IR = 3 mg·kg⁻¹·h⁻¹) through middle (IR = 6 mg·kg⁻¹·h⁻¹) to right panel (IR = 12 mg·kg⁻¹·h⁻¹), the decrease of PLE tends to be steep. However, the most abrupt recovery of PLE near ROC is observed in IR = 6 mg·kg⁻¹·h⁻¹ case.

distribution of phase difference is biased to approximately 340° (Fig. 3A). Second, the lagged relationship between the prefrontal and frontal signals persists over time (Fig. 3B). These two features lead to a dominant temporal pattern (“000”), resulting in a decrease of PLE during the unconscious state (Fig. 3C). These results are consistent with recent findings from related studies [Cimenser et al., 2011; Lee et al., 2013a; Purdon et al., 2013].

Propofol and other GABAergic anesthetics enhance GABA_A synaptic conductance and the decay time of inhibitory interneurons in the thalamus and cortex [Adodra and Hales, 1995; Ching et al., 2010]. Rich interconnection between the thalamus and prefrontal cortex [Goldman-Rakic and Porrino, 1985; Kievit and Kuypers, 1977], together with the propensity of the thalamus to generate α -oscillations [Hughes and Crunelli, 2005], promotes spatially over-coordinated activity in the α -frequency band [Lee et al., 2013a; Purdon et al., 2013]. More specifically,

increased synchronization is observed in anterior areas of the brain and is closely associated with disturbance of conscious processing [Cimenser et al., 2011; Supp et al., 2011]; although the extent to which synchronization increases is different across anesthetics [Akeju et al., 2014a,b]. Experimental and model studies suggest that this phenomenon limits flexible communication between the thalamus and the cortex, thereby promoting loss of consciousness [Ching et al., 2010; Supp et al., 2011]. In our study, the increased synchronization due to anesthesia is reinterpreted as a stereotypic communication. This new framework provided better classification of conscious states and more accurate correlation with level of consciousness.

Although current research has stressed the importance of the direction of information flow to understand anesthetic-induced unconsciousness [Boly et al., 2012; Ku et al., 2011; Lee et al., 2013b], we did not draw a conclusion about directionality between the prefrontal and

Figure 7.

PLE shows stronger agreement with the level of consciousness than do other anesthetic depth indicators ($n = 10$). (A) Representative spectrogram from a single subject during propofol sedation. Progressive decrease in peak frequency and progressive increase in $\alpha\beta$ power are shown. Corresponding MOAA/S is illustrated as black step lines. (B) BIS, RBR, ApEn, PeEn, and PLE for 6 MOAA/S scores are visualized with boxplot. In each box, the central red mark, the bottom, and the top edges indicate the median, the 25th, and 75th percentiles, respectively. Red cross marks indicate outliers. In PLE, the boxes for different MOAA/S scores overlap to a lesser degree. PLE also has less outliers compared to other measures. The P_k of PLE is higher

than those of BIS, RBR, ApEn, and PeEn; this implies that PLE has the most concordant relationship with the level of consciousness. For RBR, ApEn, PeEn, and PLE, the parameters (EEG channel, reference, and embedding parameters) which give the highest P_k were chosen (RBR: Fp1 for active channel, F8 for reference; ApEn: F4 for active channel, F7 for reference, $m=2$; and down-sampled to 128 Hz; PeEn: F4 for active channel, F7 for reference, $m=3$, and $\tau=1$; PLE: Fp1, Fp2, F3, and F4 for active channels, A2 for reference, $m=3$, and $\tau=1$). Results with different parameters are shown in Supporting Information. [Color figure can be viewed at wileyonlinelibrary.com]

frontal areas because the sign of the phase differences is not a direct indication of information flow or causality [Matias et al., 2014]. Hence we focused only on the phenomenological quantification, rather than on neurophysiological interpretation. Future studies should determine what mechanism underlies the phase relationship among cortical areas and its relevance to consciousness.

Reduced Diversity of Functional Configuration is Associated With Unconsciousness

The dynamics of PLE during anesthesia reported here are also in line with recent findings regarding consciousness. In fMRI studies, structured long-range connectivity remains in the unconscious state, while the rich repertoire of diverse functional configurations seen during consciousness is disrupted under general anesthesia [Barttfeld et al., 2014; Hudetz et al., 2015]. TMS-evoked EEG shows complex spatiotemporal patterns during the conscious state indicating integrated and informative communication among cortical areas, while stereotypic or spatially localized patterns were observed in anesthetically induced unconsciousness [Sarasso et al., 2015], sleep [Massimini et al., 2005], and coma [Casali et al., 2013]. In agreement with these observations, our results show that the functional connectivity patterns across the brain become stereotypic during propofol-induced unconsciousness.

The global neuronal workspace model postulates that prefrontal cortex plays an important role in integrating

and broadcasting distributed information [Dehaene and Changeux, 2011]. In primates, the prefrontal cortex has dense intraregion connections [Barbas and Pandya, 1989; He et al., 2007] and massive long-range fibers to other brain areas [Bassett et al., 2008; Catani and Thiebaut de Schotten, 2008]. In our results, the most prominent change due to anesthesia was found in the prefrontal EEG channels (Fp1 and Fp2). Average PLE values observed from the Fp1 and Fp2 channels were the highest among seven EEG channels during the conscious state, and are lowest during the unconscious state (Fig. 4).

PLE for Monitoring Consciousness Under General Anesthesia

Despite abundant evidence that anesthesia alters spatiotemporal dynamics of neural activity corresponding to loss of consciousness, most current DOA monitoring systems consider only temporal characteristics of single-channel EEG, thereby ignoring spatial, or connectivity information [Ferenets et al., 2007; Musizza and Ribaric, 2010]. This is partly due to the poor statistical power of strength-based average functional connectivity. According to our findings, the diversity of connectivity patterns, measured at a time scale of tens of milliseconds, more accurately tracks with the state of consciousness than does static strength-based connectivity. Second, the field spread, or volume conduction, of EEG signals [Schoffelen and Gross, 2009], which causes more than one sensor to pick up the activity of a

single source, may hinder the application of connectivity information to DOA monitoring systems. We mitigate the field spread problem through the symbolization of the phase lead-lag relationship; the imaginary components of cross-spectra, or the sign of phase difference are relatively robust to the field spread problem [Nolte et al., 2004; Stam et al., 2007]. As a result, PLE is less influenced by the choice of reference channel (Supporting Information, Fig. 3).

Among the four DOA indicators studied, PLE exhibited the highest agreement with the level of consciousness measured with MOAA/S. The BIS, RBR, ApEn, and PeEn assess only temporal features of a single-channel EEG and therefore dismiss spatial relationships across cortical areas, which have critical relevance to the states of consciousness according to recent findings [Casali et al., 2013; Cimenser et al., 2011; Supp et al., 2011]. In addition to its classification and correlation performance, PLE is mathematically simple, computationally efficient, and highly robust to the noise and the choice of reference, thereby showing promise as a basis for consciousness or anesthetic depth monitoring system.

Limitations

In our analysis, a low PLE value results from the existence of prolonged symbols as in the artificial symbol sequence of Figure 1A; that is, the patterns “000” or “111” were dominant. In real data, it is very unlikely that patterns “010” and “101” contribute to decrease PLE, as in Figure 1C. Usually, high PLE is obtained if phase differences change sign frequently; that is, the occurrence of patterns “010” and “101” tends to increase PLE. This is most common when the phase difference is distributed around zero. Unfortunately, it remains unclear whether the high PLE in the conscious state results from genuine flexible interaction or from the field spread of EEG signal. However, it is very unlikely that the persistent phase-lag relationship between the prefrontal and frontal areas during the unconscious states, in which the phase difference was located approximately around 340° (Fig. 3A), results from field spread. As the quasi-static approximation holds for EEG [Stinstra and Peters, 1998], the phase delay due to field spread is approximately zero or 180° . Moreover, the PLE results were very robust to the choice of reference, thereby advocating our hypothesis.

Although PLE shows very good agreement with the level of consciousness, it exhibits the opposite trend at higher concentrations of propofol. PLE in A3, which is a deeper stage of anesthesia, is higher than PLE in A2 (Fig. 4), and further increases as anesthesia continues. In this sense, PLE may not serve as a universal measure of consciousness. Moreover, the low PLE during the unconscious state is closely related to the frontal synchronization induced by propofol. It is unclear how it will change in other altered states of consciousness, such as non-

GABAergic anesthesia, sleep, or coma, where there is no such consistent increase in frontal synchrony. Further research should investigate how the diversity of the phase-lag pattern relates to other altered states of consciousness.

CONCLUSION

Using the temporal dynamics of the phase relationships of multichannel EEG, PLE can successfully differentiate the states of, and the level of, consciousness. The lack of diversity in functional connectivity patterns during propofol-induced unconsciousness supports recent theories and experiments regarding consciousness. The proposed measure and results may improve clinical assessment of consciousness during anesthesia.

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DISCLOSURE

The authors declare no competing interests. Drs Lee, Noh, Jung, and Kim hold a patent (pending) in Korea and US through InBody Co., Pohang University of Science and Technology, and University of Ulsan College of Medicine (Korea: Application No. 10-2015-0188334, Filed December 29, 2015; US: Application No. 15391412, Filed December 27, 2016; “Methods and apparatus for monitoring consciousness”).

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