ANESTHESIOLOGY

Dynamic Cortical Connectivity during General Anesthesia in Healthy Volunteers

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EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- Anesthetic-induced loss of consciousness is accompanied by changes in functional connectivity within and between brain networks.

What This Article Tells Us That Is New

- Despite a stable surgical level of anesthesia and the absence of noxious stimuli, connectivity patterns are not static but rather fluctuate dynamically and nonrandomly over time. These results suggest that single or static connectivity patterns may not be able to discriminate levels of consciousness.

ABSTRACT

Background: Recent studies of anesthetic-induced unconsciousness in healthy volunteers have focused on functional brain connectivity patterns, but the protocols rarely parallel the depth and duration of surgical anesthesia. Furthermore, it is unknown whether there is a single functional connectivity pattern that correlates with general anesthesia for the duration of prolonged anesthetic exposure.

Methods: The authors analyzed electroencephalographic data in 30 healthy participants who underwent induction of anesthesia with propofol followed by 3 h of isoflurane anesthesia at age-adjusted 1.3 minimum alveolar concentration. Functional connectivity was assessed by frequency-resolved weighted phase lag index between frontal and parietal channels and between prefrontal and frontal channels, which were classified into a discrete set of states through k-means cluster analysis. Temporal dynamics were evaluated by the occurrence rate and dwell time distribution for each state as well as the transition probabilities between states.

Results: Burst suppression was present, with mean suppression ratio reducing from 44.8 ± 32.3% to 14.0 ± 20.2% (mean ± SD) during isoflurane anesthesia ($P < 0.001$). Aside from burst suppression, eight connectivity states were classified by optimizing the reproducibility of clustering solutions, with each characterized by distinct properties. The temporal progression of dominant states revealed a successive shifting trajectory from the state associated with alpha frontal-parietal connectivity to those associated with delta and alpha prefrontal-frontal connectivity during induction, which was reversed during emergence. Cortical connectivity was dynamic during maintenance period, and it was more probable to remain in the same state (82.0 ± 8.3%) than to switch to a different state ($P < 0.001$). However, transitions to other states were structured, *i.e.*, occurred more frequently than expected by chance.

Conclusions: Anesthesia-induced alterations of functional connectivity are dynamic despite the stable and prolonged administration of isoflurane, in the absence of any noxious stimuli. Changes in connectivity over time will likely yield more information as a marker or mechanism of surgical anesthesia than any single pattern.

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Recent studies of anesthetic-induced unconsciousness have focused on functional brain dynamics and connectivity patterns.[1] Specifically, the functional disconnection of frontal cortex from posterior regions has been associated with unconsciousness induced by diverse anesthetics, despite different molecular and neurophysiologic properties.[2–6] Long-range connectivity can be disrupted despite local increases in coherence,[7] most notably alpha coherence in the anterior cortex.[8,9] These changes in cortical connectivity could be a drug-invariant marker for discriminating unconscious from conscious states during anesthesia. However, most studies of anesthetic-induced unconsciousness in healthy volunteers have typically employed anesthetic protocols that can be characterized as either "long but light" (just across the threshold of unresponsiveness) or "deep but brief" (more profound unconsciousness but for a brief period of time). These conditions are not the same as general anesthesia for major surgery, which typically requires prolonged periods

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of anesthetic exposure at concentrations far higher than those administered to induce unconsciousness.[10]

Although certain consistent effects of anesthesia on functional connectivity have been observed in spontaneous brain activity,[11] several key questions remain. First, it is unclear if the same connectivity patterns are still observed at clinically relevant anesthetic concentrations, but without the confound of surgical intervention. Second, it is unknown if the patterns are static or dynamic during the prolonged period of anesthetic exposure at concentrations required for major surgery. Third, it is unknown if connectivity patterns in humans undergo metastable state changes as has been found to occur with electroencephalographic dynamics in rodents during prolonged anesthesia.[12,13] Metastability refers to states that fall outside of a stable equilibrium but nonetheless persist for an extended period of time and shift in structured ways.

To address these questions, we analyzed multi-channel electroencephalographic data in 30 healthy participants who underwent induction of anesthesia with propofol followed by 3 h of age-adjusted 1.3 minimum alveolar concentration (MAC) of isoflurane anesthesia. There were two primary objectives in this study. The first was to characterize the temporal progression of cortical connectivity during anesthesia-induced alterations of consciousness. Previous studies typically focused on the analysis of averaged connectivity across selected epochs spanning several minutes that represent depressed consciousness induced by general anesthesia, but the temporal variations within the epochs of analysis and the evolution between the discrete epochs of analysis have not been widely investigated. The second objective was to focus on the prolonged maintenance period and test if cortical connectivity is static or dynamic during this pharmacologically steady state period, which may provide evidence for metastability in humans during general anesthesia.

Materials and Methods

In this study, we analyzed electroencephalographic data in 30 healthy volunteers who underwent general anesthesia in the Reconstructing Consciousness and Cognition (ReCCognition) study (NCT01911195).[14] This multicenter study was reviewed and approved by the Institutional Review Board specializing in human subjects research at the University of Michigan, Ann Arbor, Michigan (HUM0071578), University of Pennsylvania, Philadelphia, Pennsylvania (818401), and Washington University in St. Louis, Missouri (201308073). Ten healthy volunteers at each of the three study sites were recruited using posted flyers and written informed consent was obtained from all participants after careful discussion.

Study Population

As previously described,[10] participants were American Society of Anesthesiologists class I physical status, 20 to 40 yr of age, body mass index less than 30 kg/m^2, with Mallampati I or II airway classification, and no other factors predictive of difficult airway. We excluded subjects who were pregnant, had a history of obstructive sleep apnea, reactive airway disease, neurologic or psychiatric disease/history, history or current use of psychotropic medications, gastroesophageal reflux, cardiac conduction abnormalities, history of problems with anesthesia, or family history of problems with anesthesia. Pregnancy and illicit drug use were ruled out through urine and blood analyses.

Experimental Protocol

The full protocol for the ReCCognition study can be found in previous publications.[10,14] In this study, we investigated cortical connectivity changes during exposure to general anesthesia. First, a 5-min, eyes-closed electroencephalographic baseline period was obtained prior to anesthetic induction. The induction of anesthesia was then conducted with IV propofol at increasing infusion rates during three consecutive 5-min blocks (block 1: 100 $\mu g \cdot kg^{-1} \cdot min^{-1}$; block 2: 200 $\mu g \cdot kg^{-1} \cdot min^{-1}$; block 3: 300 $\mu g \cdot kg^{-1} \cdot min^{-1}$), with loss of consciousness as assessed by loss of responsiveness to an audio command to squeeze left or right hands (in random order). Isoflurane was then administered with air and 40% oxygen at a constant 1.3 age-adjusted MAC *via* laryngeal mask airway. After 3 h of exposure to isoflurane, the anesthetic was discontinued and participant responsiveness was assessed every 30 s until the recovery of consciousness (see previously published protocol[14]). We then conducted a 5-min, eyes-closed electroencephalographic rest period 30 min after recovery of consciousness.

Electroencephalographic Analysis

Data Acquisition and Preprocessing

The electroencephalographic data were acquired with the 32-channel EGI HydroCel system (Electrical Geodesics, Inc., USA) for each participant enrolled at the University of Pennsylvania and University of Washington in St. Louis; a 128-channel system was used at the University of Michigan. The electroencephalographic signals were digitized continuously at 500 Hz with a vertex reference. The raw signals were exported into MATLAB (version 2017a; MathWorks, Inc., USA) and down-sampled to 250 Hz. Electrodes on the lowest parts of the face and head were removed, leaving 21 (or 90 for 128-channel recordings) channels on the scalp. For conformity, the same 21 channels were taken from all participants and used for the analysis.

The electroencephalographic recordings were preprocessed as follows. First, the signals were detrended using a local linear regression method with a 10-s window at a step size of 5-s in Chronux analysis toolbox (version 2.11; http://chronux.org/. Accessed August 18, 2017.)[15] Data were then re-referenced to linked-mastoids; due to bad channels, only the right or left mastoid was used as reference in 5 of the 30

participants. Second, noisy data segments were detected and rejected in a stepwise manner. The signals were bandpass filtered at 0.5 to 55 Hz *via* a 5-order Butterworth filter using a zero-phase forward and reverse algorithm and then divided into 2-s windows. The 2-s data were rejected if (1) the average amplitude was greater than 4 average amplitude (or its SD greater than 2 SD value) of the whole recording, and (2) the above was present in at least 4 of the 21 channels. This step was performed for the baseline, anesthesia, and recovery periods separately, and 7.0 ± 5.4%, 6.1 ± 2.1%, 17.3 ± 13.2% (mean ± SD) of the data were rejected, respectively. Third, electroencephalographic data from baseline and recovery periods were visually inspected, and independent component analysis was applied to remove the components representing eye movement, and muscle or movement artifacts in four participants during baseline and eight participants during recovery periods, using the extended-Infomax algorithm in EEGLAB toolbox.[16]

For each participant, the preprocessed signals during baseline, anesthesia, and recovery were concatenated for the analysis, as illustrated in figure 1A. For the anesthetic period, the electroencephalographic signals from propofol infusion to recovery of consciousness were included in the analysis. The induction time (from the start of propofol infusion to loss of consciousness) was 10.5 ± 2.9 min across all participants. Loss of consciousness was achieved during propofol infusion in 27 participants, but only after the administration of isoflurane in three participants. The electroencephalographic recordings were temporarily interrupted in two participants and the emergence time (from the discontinuation of isoflurane to recovery of consciousness) was 39.6 ± 13.4 min (N = 28).

Quantification of Burst Suppression

The electroencephalographic recordings showed burst suppression during anesthetic exposure. The presence of burst suppression precludes a direct analysis of cortical connectivity, since the analysis windows do not meet the requirement of stationarity, and the resultant connectivity may conflate the high and low values contributed by burst and suppression episodes, respectively. To resolve this issue, we followed our previous study[17] for the quantification of burst suppression. Specifically, the spectral analysis was first performed with the short-time Fourier transform to provide information of frequency content of burst and suppression episodes in the electroencephalographic signals. The data were then transformed in three steps: (1) signals were bandpass filtered at 5 to 30 Hz *via* a 4-order Butterworth filter; (2) the Hilbert transform of the bandpassed signal was used to calculate the instantaneous amplitude, which was further smoothed with a moving average filter of 0.5 s; and (3) a threshold calculated from the manually labeled suppression periods (mean + 4 SD) was applied to the transformed signal to yield a binary series of burst and suppression states. In this study, the minimum length of burst and suppression episodes was set to 0.5 s,[18] and the suppression ratio was calculated as percentage of time spent in suppression of each 30-s binary series at a step size of 10 s. The above analysis was applied to the electroencephalographic data from propofol infusion to 5 min after the discontinuation of isoflurane. The suppression ratio was calculated for each channel and then averaged across the channels. To evaluate the temporal progression of burst suppression, we calculated the mean suppression ratio value for each consecutive 30-min segment during the 3-h exposure of isoflurane. For the dynamic connectivity analysis, we defined the presence of burst suppression for a certain time window if the suppression ratio was greater than 20% and these windows were separated and excluded from the subsequent connectivity analysis.

Estimation of Functional Connectivity

The functional connectivity was estimated using weighted phase lag index.[19] This is a measure of phase synchronization that is relatively resistant to the effects of volume conduction and reference montage by accounting for only nonzero phase lead/lag relationships.[19,20] Given two signals x_i and x_j, the weighted phase lag index (*wPLI*) is defined as[19]

$$wPLI_{ij} = \frac{\left|E\left\{\Im\left(C_{ij}\right)\right\}\right|}{E\left\{\left|\Im\left(C_{ij}\right)\right|\right\}} = \frac{\left|E\left\{\left|\Im\left(C_{ij}\right)\right| \cdot sgn\left(\Im\left(C_{ij}\right)\right)\right\}\right|}{E\left\{\left|\Im\left(C_{ij}\right)\right|\right\}}$$

where $\Im(C_{ij})$ is the imaginary part of cross-spectrum C_{ij} between x_i and x_j, $E\{\cdot\}$ is the expected value operator, $|\cdot|$ is the absolute value function, and $sgn(\cdot)$ is the sign function. The weighted phase lag index values range between 0 (no locking) and 1 (perfect locking). If the phases of x_i always lead or lag those of x_j, the $wPLI_{ij}$ equals to 1; alternatively, if the phase lead/lag relationship of two signals is random, the $wPLI_{ij}$ value will be low. If there is no phase difference between the two signals, the $wPLI_{ij}$ value will then be 0.

For implementation, the 21-channel electroencephalographic signals during baseline, anesthesia, and recovery were divided into 30-s windows at a step size of 10 s, and for each window, the weighted phase lag index as a function of frequency was estimated between each pair of channels using a custom-written function adapted from Fieldtrip toolbox.[21] Specifically, the bivariate time series, x_i and x_j, were divided into K sub-windows of 2-s with 50% overlap; for the time series in each sub-window k ($k = 1,2,\ldots,K$), x_i^k and x_j^k, its cross-spectrum, C_{ij}^k, was estimated as a function of frequency using the multitaper method with time-bandwidth product = 2, spectral resolution = 2 Hz, and number of tapers = 3.[15] From the K estimations of cross-spectra, the $wPLI_{ij}$ values were calculated at variable frequencies according to the equation. To mitigate the potential bias of $wPLI_{ij}$, a pair of surrogate data were generated by the trial-shuffling method, *i.e.*, randomly shuffling the index of one time series (x_i^m, $m = 1,2,\ldots K$, $m \neq k$), while keeping the other signal (x_j^k) unchanged; the cross-spectrum and the weighted phase lag index was calculated with these

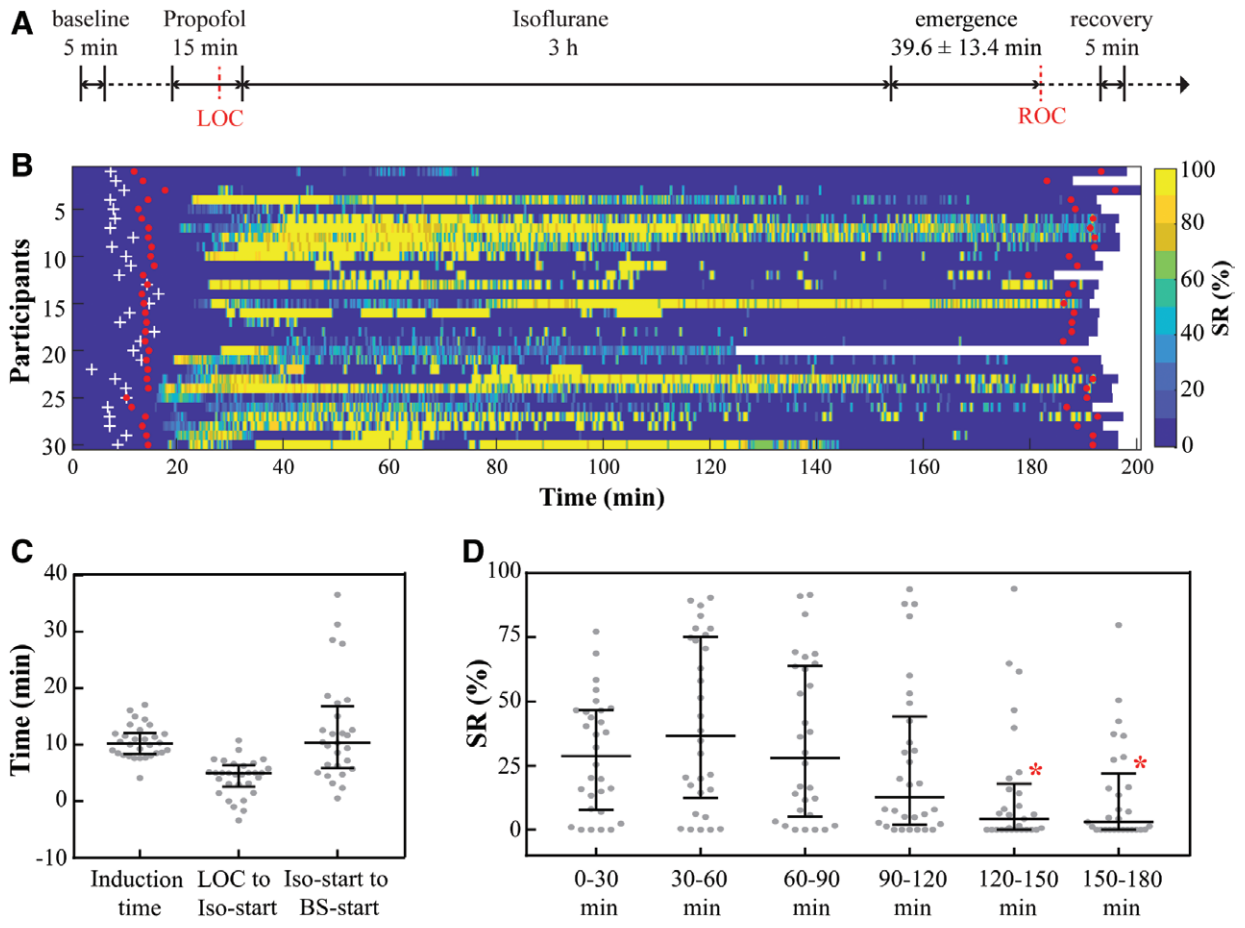

Fig. 1. Experimental design and the quantification of electroencephalographic burst suppression. (*A*) Experimental design and timeline. The electroencephalogram was recorded throughout the entire experiment, and the three periods during baseline, anesthesia and recovery, as indicated in *bold horizontal lines*, were concatenated for the analysis. (*B*) The time courses of suppression ratio (SR) from propofol infusion to 5 min after the discontinuation of isoflurane for individual participants. For each participant, the time of loss of consciousness (LOC) was marked in *white cross*, while the start and end time of isoflurane exposure were labeled in *red dots*. The electroencephalographic recording was temporarily interrupted before the discontinuation of isoflurane in participant 20. (*C*) The duration of induction time (from propofol infusion to LOC), the time from LOC to the start of isoflurane administration (Iso-start), and the elapsed time from the isoflurane administration to the start of burst suppression (BS-start, defined as the first time window with SR greater than 20%). (*D*) The mean SR value for each consecutive 30-min segment during the 3-h isoflurane exposure period. In (*C* and *D*), the *gray dots* represent the individual data, *horizontal lines* represent median, and error bars represent the interquartile range. *Indicates adjusted $P < 0.05$ *versus* the 0 to 30 min, 30 to 60 min, 60 to 90 min, and 90 to 120 min segments by Friedman test followed by *post hoc* Dunn tests. ROC, recovery of consciousness.

shuffled data pairs, which was subtracted from the original weighted phase lag index. This produced the final estimation of functional connectivity.[22]

In this study, we focused on select cortical regions: frontal-parietal connectivity as the averaged weighted phase lag index across the combinations of F3, F4, Fz and P3, P4, Pz, and prefrontal-frontal connectivity as the averaged weighted phase lag index between Fp1, Fp2, Fpz and F3, F4, Fz. We chose these cortical regions because of their relevance to anesthetic-induced unconsciousness based on our previous studies.[2,3,10,23] We have also confirmed that cortical connectivity changes across altered conscious states were mainly concentrated on the chosen regions (fig. S1, Supplemental Digital Content 1, http://links.lww.com/ALN/B885).

Dynamic Connectivity Analysis

To explore the fluctuations of cortical connectivity during anesthesia, the frequency-resolved frontal-parietal and prefrontal-frontal connectivity was estimated at each time window for each participant, while excluding windows in which burst suppression was present. The connectivity pattern obtained was a 140-dimensional vector, with each pattern including 70 frequency estimates for 0.5 to 35 Hz. These data were then aggregated across all participants and subjected to principal component analysis. Principal

component analysis exploits the covariance structure of the variables (140 variables in this study), to identify mutually orthogonal directions (*i.e.*, principal components) along which most of the fluctuations occur. By principal component analysis, we reduced the original 140-dimensional pattern to *M*-dimensional feature, while maximally preserving the amount of variance from the original connectivity pattern. The *M*-dimensional connectivity features were then classified into N_c clusters using k-means clustering algorithm with squared Euclidean distance and 100 replications of the initial centroids. In this study, the number of clusters (N_c), together with the number of retained principal components (*M*), was determined by the stability index that quantifies the reproducibility of clustering solutions for the studied dataset,[12,24] the amount of explained variance by the retained principal components, and the interpretability of the clustering results (Supplemental Digital Content 2, http://links.lww.com/ALN/B886).

Dynamic Cortical Connectivity during Anesthesia-induced Alterations of Consciousness

The cluster analysis partitioned each non–burst suppression time window into one of the N_c clusters. Each cluster can be regarded as a connectivity state that is characterized by distinct spectral and spatial properties. Based on the squared Euclidean distance with these characterized patterns, each time window can be assigned a unique state label; for the windows with burst suppression, we classified them into an additional state as "BS." The connectivity state time series for consecutive time windows for each participant was thus obtained, which represents the evolution of connectivity states during alterations of consciousness induced by general anesthesia.

To characterize the temporal dynamics of the connectivity states, we quantified the occurrence rate and dwell time distribution for each state. The former is defined as the fraction of time spent in each connectivity state among all the states, while the latter measures the distribution of the temporal duration of state visits. Furthermore, to assess how cortical connectivity shifts over time, we calculated the distribution of participants across the $N_c + 1$ states (*i.e.*, the percentage of participants in each state) at each time window, by rescaling the time spans during baseline, induction, maintenance (from loss of consciousness to the discontinuation of isoflurane), emergence, and recovery across all participants. Shannon entropy was used to quantify this distribution, with lower values indicating the co-occurrence of a dominant state in most of the participants and higher values indicating the presence of multiple states across the participants at that time window.

Connectivity State Transitions during Isoflurane Maintenance

We further focused on the maintenance period and investigated whether cortical connectivity is static or whether it transitions among different states during the stable period from 30 min after isoflurane administration to the discontinuation of isoflurane, with an assumption that the propofol effect was minimized by that time and the isoflurane reached pharmacologic steady state conditions during this period. Following the study,[12] we analyzed the connectivity state time series as a Markov chain (*i.e.*, the state transition depends only on the current state). For each participant, we first counted the times they stayed in a certain brain connectivity state (defined as the cortical connectivity at time $t + 1$ remaining within the same state as time t), as well as the number of transitions to other states. Based on previous studies,[12,13] we hypothesized that cortical connectivity would be persistent or "sticky" in the same state. We also hypothesized that transitions of state, with the possibility of switching to any of the other states, will be much less frequent and uneven across states and participants. To this end, instead of assessing the probability of state transitions at both the state and participant level, we performed the analysis in two alternative ways. First, at a participant level, we aggregated over all states and obtained the times of staying in any state, i ($i = 1, 2, \ldots, N_c + 1$) and that of switching to any of the other states, j ($j = 1, 2, \ldots, N_c + 1$, but $j \neq i$). We then calculated the probabilities of state stays and state switches by dividing the total times of state stays and switches for each participant. Second, following previous studies,[12,25–28] we calculated the transition probability for each pair of connectivity states by using all participants. Transition probability measures the likelihood of the current state transiting to another state at a future time at the group level. Specifically, for the transition from State i to State j ($i, j = 1, 2, \ldots, N_c + 1$), the probability was estimated by counting the times of this transition divided by the total times of all state transitions (including stays and switches) across all participants; the matrix so obtained represented the transition probability for each pair of connectivity states, with all elements of the matrix summing up to 1.

Statistical Analysis

This exploratory study was performed on an existing dataset of 30 participants who underwent general anesthesia in the ReCCognition study. No *a priori* statistical power calculation was conducted specifically for this study; however, past studies of functional cortical connectivity during general anesthesia have successfully detected significant changes in substantially fewer participants.[3,4,29] If not specifically stated, statistical analyses were performed using MATLAB, and data were presented in the form of median with interquartile range in figures and in the form of mean ± SD in the text for readability. All data sets were tested for normality of distribution by Lilliefors corrected Kolmogorov–Smirnov tests.

To assess temporal changes in burst suppression during isoflurane anesthesia, statistical comparisons were performed on the mean suppression ratio values across the six 30-min segments by using the Friedman test followed by *post hoc* Dunn multiple comparison tests (Prism 7.03, Graphpad Software, Inc., USA), with the adjusted $P < 0.05$ considered significant. Similarly, the Friedman test followed by *post hoc* Dunn tests was used to evaluate the difference in

the occurrence rate and number of state transitions across connectivity states, respectively. Before the tests, the null hypothesis of normality of distribution was rejected in most of the data sets ($P < 0.05$).

To compare the likelihood of remaining in the same state or switching to a different state, Wilcoxon signed-rank test was used to compare the probabilities of state stays and state switches across the participants. To test the statistical significance of state transitions between each pair of connectivity states, we performed the following surrogate data analysis. First, N = 1,000 surrogate time series were generated by randomly shuffling the connectivity state time series for each participant, which permutated the temporal order of the state occurrence while keeping the occurrence rate of the states. Second, we focused on between-state transitions[25,27,28] and generated N = 1,000 surrogate time series by randomly permutating the retained state time series (including only state switches) after removing the state stays, with the constraint of each state's occurrence rate across all participants. With each surrogate time series, the transition probability was calculated for each pair of connectivity states, and a state transition was deemed statistically significant by comparing the original transition probability with those from surrogate data. The significance value was obtained through the cumulative distribution function, $p = 1 - \int_{-\infty}^{\alpha} p_{surro}(h)\,dh$, where α denotes the original transition probability and $p_{surro}(h)$ denotes the estimated normal distribution if the null hypothesis of normality of surrogate data could not be rejected, or empirical surrogate distribution otherwise.[30] Across all the studied state transitions, the false discovery rate–adjusted $P < 0.05$ was considered significant.

Results

Burst Suppression Patterns during Isoflurane Anesthesia

Burst suppression was induced during the prolonged period of isoflurane exposure. The pattern was present in all but two participants and there was considerable participant-to-participant variability in terms of the start time, duration, and the suppression level as measured by suppression ratio (fig. 1B). The pattern started after 12.5 ± 9.1 (N = 28) min from the administration of isoflurane (fig. 1C), which was typically intermittent with continuous electroencephalographic signals, with the duration of 78.6 ± 51.6 (N = 27) min across participants. The mean suppression ratio values were not constant during the 3 h of isoflurane anesthesia ($\chi^2[6,27] = 57.5$; $P < 0.001$). The electroencephalographic signals were largely suppressed during the 30 to 60 min of isoflurane exposure, with the mean suppression ratio of 44.8 ± 32.3%, which was then gradually weakened, with the mean suppression ratio reducing to 15.6 ± 24.5% and 14.0 ± 20.2% for the last hour of isoflurane exposure (120- to 150-min segment: $P = 0.003$ *vs.* 0 to 30 min: $P < 0.001$ *vs.* 30 to 60 min and 60 to 90 min: $P = 0.043$ *vs.* 90- to 120-min segment; 150- to 180-min segment: $P = 0.002$ *vs.* 0 to 30 min: $P < 0.001$ *vs.* 30 to 60 min and 60 to 90 min: $P = 0.034$ *vs.* 90- to 120-min segment; fig. 1D). To focus on connectivity and cluster analysis, we separated burst suppression time windows accordingly, then pooled these time windows with the classified connectivity states for the characterization of the temporal dynamics of brain activity during anesthesia.

Dynamic Cortical Connectivity during Anesthesia-induced Alterations of Consciousness

For each participant, the weighted phase lag index-based connectivity pattern was estimated for each non–burst suppression window, which was then subjected to principal component analysis for dimensionality reduction. The first seven principal components were retained and then classified into eight clusters, while the time windows with burst suppression were assigned to an additional cluster "BS" (fig. 2). Figure 3 shows the individual time courses of connectivity states for all participants. The retained seven principal components contained 62.3% of the total variance of the original connectivity patterns (fig. S3, Supplemental Digital Content 2, http://links.lww.com/ALN/B886). The stability index was 0.23 ± 0.07 with the seven retained principal components and eight clusters, or equivalently the 1-minimum Hamming distance was 0.80 ± 0.06 across the different solutions, which suggests that 80% of the data were allocated to the same clusters through different clustering solutions and the clustering was 80% consistent among participants.

Each cluster (*i.e.,* connectivity state) demonstrated its characteristic connectivity pattern with distinct spatial (frontal-parietal and prefrontal-frontal) and spectral (delta, theta, alpha, and high frequencies) properties (fig. 4A and table 1; figs. S4 and S5, Supplemental Digital Content 2, http://links.lww.com/ALN/B886, which demonstrate the extent to which these clusters were separated). The temporal characteristics of these connectivity states were assessed by the occurrence rate (fig. 4B) and dwell time distribution (fig. 4C) for each state. There was no significant difference in the occurrence rate across the eight non–burst suppression states ($P > 0.999$), and no state was specific to a particular participant or a subgroup of participants. On the other hand, the dwell time varied from 30 s to a few minutes among multiple visits to a certain state. Across all non–burst suppression states, the dwell time was longer than 30 s for 68.1 ± 3.9% of the state visits, among which 25.0 ± 3.4% were longer than 60 s. Taken together, these results suggest the existence of multiple connectivity states during anesthesia in individual participants.

Figure 4D further presents the temporal progression of connectivity state distributions across participants. At baseline, the alpha frontal-parietal connectivity (State 1) was dominant, which decreased with the induction of propofol and shifted toward State 2 with a prominent 10 to 20 Hz

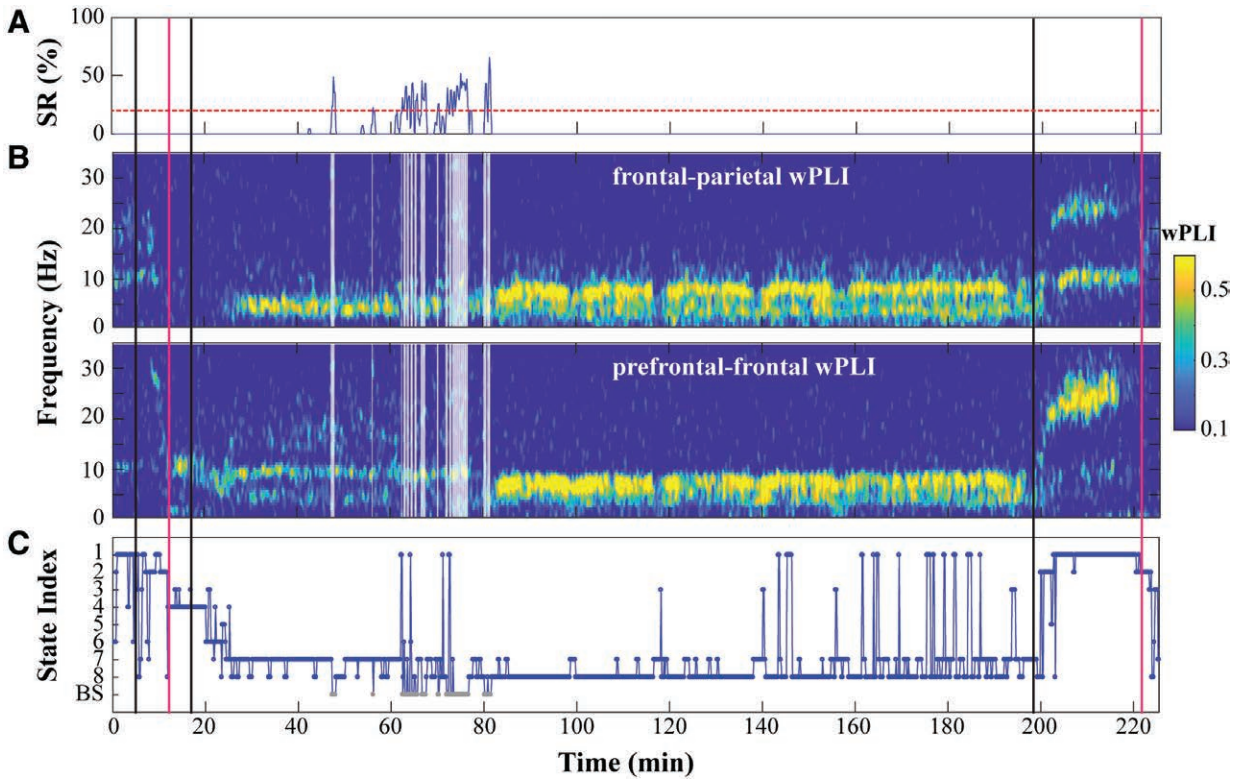

Fig. 2. The dynamic connectivity analysis method. (*A*) Representative time plot of suppression ratio (SR), (*B*) frontal-parietal and prefrontal-frontal connectograms, and (*C*) time course of connectivity states from cluster analysis. The connectivity patterns (*i.e.,* frontal-parietal and prefrontal-frontal weighted phase lag index [wPLI]) from all non–burst suppression windows were subjected to principal component analysis for dimensionality reduction and then classified into eight clusters (*blue circles*) *via* k-means clustering, while the windows with burst suppression were partitioned into an additional cluster "BS" (*gray circles*). The *black vertical lines* indicate the start of propofol infusion, the start and end of isoflurane exposure from left to right, and the *red vertical lines* indicate the time of loss of consciousness and recovery of consciousness, respectively.

frontal-parietal and a mild 10 to 30 Hz prefrontal-frontal connectivity before loss of consciousness. State 3 demonstrated a predominance of delta connectivity, which was observed right after loss of consciousness. Connectivity then shifted to alpha prefrontal-frontal connectivity (State 4). The dominance of State 4 was also reflected by the dramatically reduced entropy value (as low as 0.41) that suggests the co-occurrence of State 4 in most participants. Burst suppression was dominant with the chosen MAC value of isoflurane; otherwise, there was no single dominant state, while four states existed with varied spatial and spectral (delta, theta, alpha) properties, with the averaged entropy value of 0.85 for the last hour of the maintenance period. The dominant pattern returned to State 2 for the majority of the emergence period and then to State 1 after recovery. The group-level analysis revealed a shifting trajectory of dominant states during anesthesia-induced alterations of consciousness. Furthermore, the existence of several connectivity states suggests that the cortical connectivity is dynamic during the pharmacologically steady state maintenance period.

Connectivity State Transitions during Isoflurane Maintenance

We investigated how cortical connectivity transitions among different states during a prolonged maintenance period of general anesthesia. Despite the variability in state occurrence rate, the times of staying in each of the connectivity states was higher than that of switching to any of the other states (fig. S6, Supplemental Digital Content 3, http://links.lww.com/ALN/B887). It is expected that the times of state switches were low and variable across states and participants. Aggregated over all connectivity states, the cortical connectivity was sticky in the same state, with the probability of 82.0 ± 8.3%, which was significantly higher than that of switching to a different state (18.0 ± 8.3% across all participants [$P < 0.001$]). Alternatively, the group-level transition probability matrix using all participants presents the probability of state transitions for each pair of connectivity states (fig. 5A). It is visibly evident that the persistence in the same state was more probable than switching to a different state. Relative to random transitions by permutating

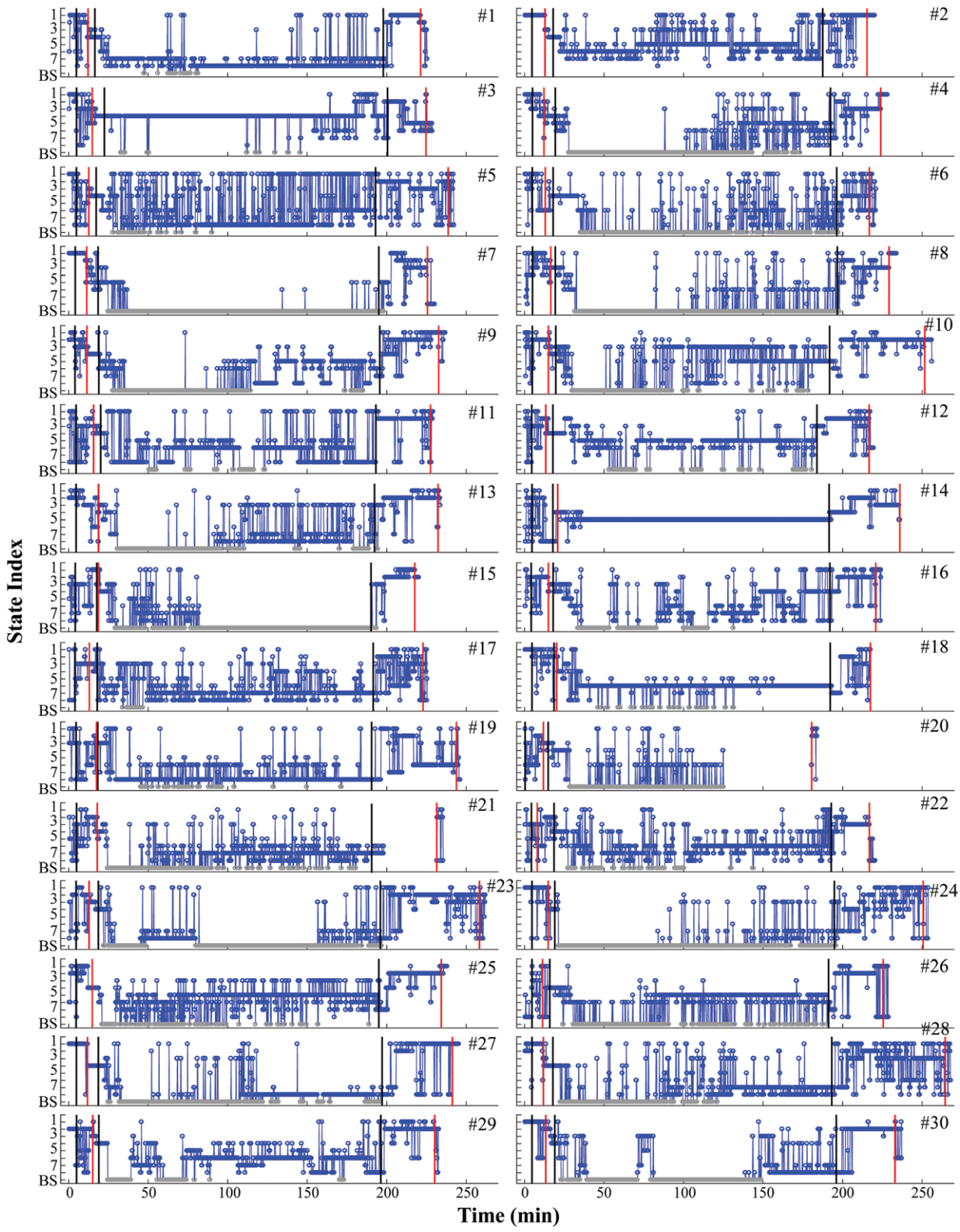

Fig. 3. Individual time courses of connectivity states from cluster analysis. The *black vertical lines* indicate the start of propofol infusion, the start and end of isoflurane exposure from left to right, and the *red vertical lines* indicate the time of loss of consciousness and recovery of consciousness, respectively. The electroencephalographic recording was temporarily interrupted in participants 20 and 21. The baseline period was not recorded in 20, and the recovery period was not recorded in 14 and 18. BS, burst suppression.

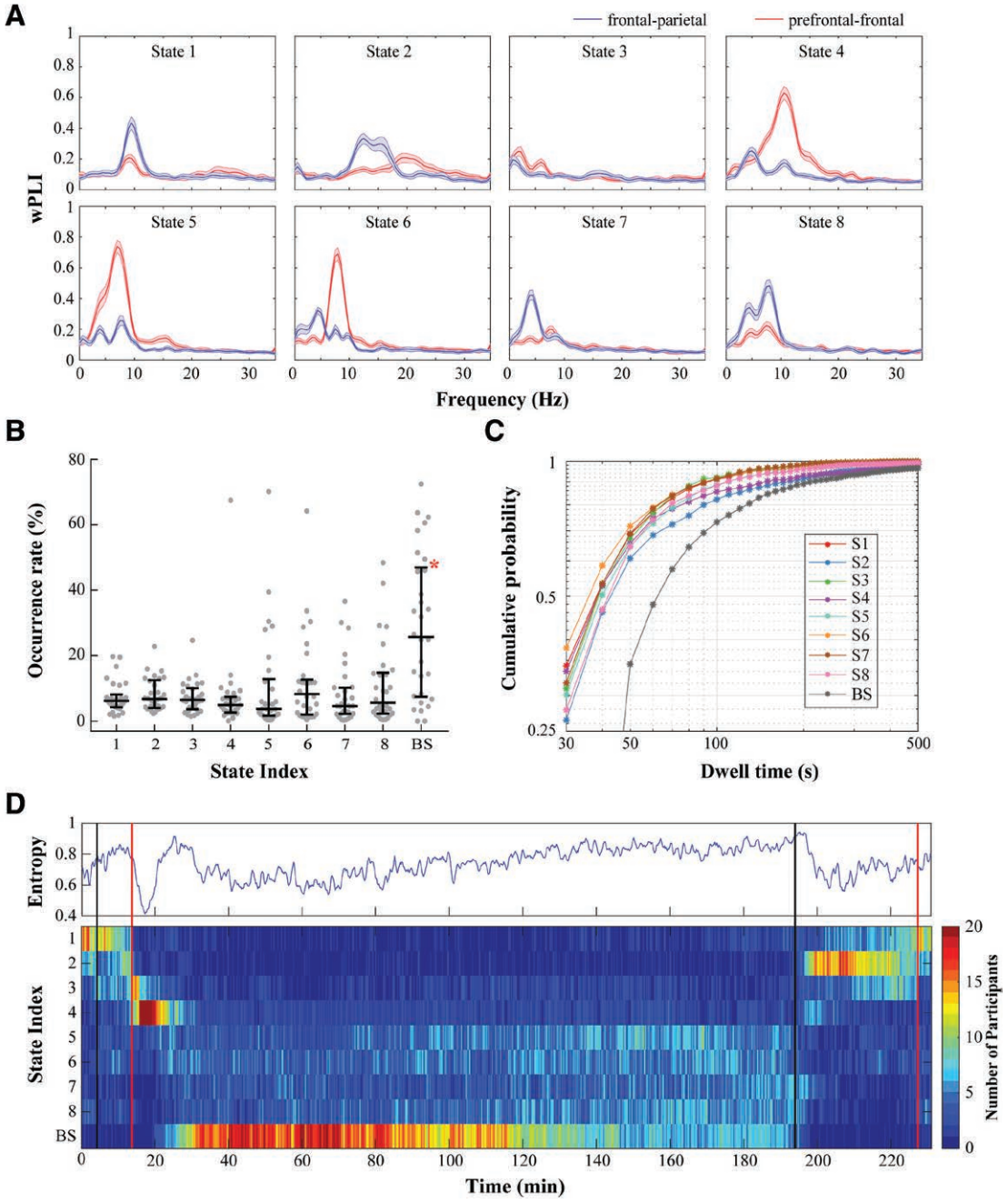

Fig. 4. The dynamic cortical connectivity during anesthesia-induced alterations of consciousness. (*A*) Representative connectivity patterns, *i.e.*, the mean and its 95% CI (*blue*: frontal-parietal weighted phase lag index [wPLI]; *red*: prefrontal-frontal wPLI), which were obtained from the 100 samples closest to the centroid for each cluster. (*B*) Occurrence rate, *i.e.*, the fraction of time spent in each cluster (connectivity state), with the *gray dots* representing the individual data, *horizontal lines* and error bars representing median and the interquartile range across all participants. *Indicates adjusted $P < 0.05$ *versus* States 1, 3, and 8; $P < 0.001$ *versus* States 4, 5, and 7 by Friedman test followed by *post hoc* Dunn tests. (*C*) Cumulative dwell time for each connectivity state across all participants. (*D*) The temporal progression of the distribution of the participants across the connectivity states, *i.e.*, the percent of participants in each state. From left to right, the first *vertical line in black* indicates the start of propofol infusion that separates the baseline and induction periods, the first *red line* indicates the time where loss of consciousness occurred, the second *black line* indicates the discontinuation of isoflurane, and the second *red line* indicates recovery of consciousness that separates emergence and recovery periods. The time spans during baseline, induction, maintenance, emergence, and recovery were rescaled before the calculation. On the *top*, Shannon entropy was calculated to quantify the distribution of participants over time, with the lower values indicating a dominant state, while higher values indicating more distributed across multiple states. BS, burst suppression; S, state.

Table 1. Characteristics of the Connectivity States

Connectivity State	Dominant Frequency Range	Predominant Region	Associated Conscious State
State 1	7–13 Hz	Frontal-parietal	Wakefulness
State 2	10–20 Hz	Frontal-parietal	Induction and emergence
State 3	0.5–4 Hz	Prefrontal-frontal	Right after LOC
State 4	6–15 Hz	Prefrontal-frontal	After LOC, before BS
State 5	3–10 Hz	Prefrontal-frontal	Maintenance
State 6	6–10 Hz	Prefrontal-frontal	Maintenance
State 7	2–6 Hz	Frontal-parietal	Maintenance
State 8	3–10 Hz	Frontal-parietal	Maintenance
BS	Burst suppression	--	Maintenance

BS, burst suppression; LOC, loss of consciousness.

the temporal order while keeping the occurrence rate of the states, the persistent probabilities were significantly higher ($P < 0.05$), suggesting that cortical connectivity is more likely to be sticky than expected by chance (figs. 5B and S7, Supplemental Digital Content 3, http://links.lww.com/ALN/B887). Furthermore, we focused on the between-state transitions and compared the original transition probability with those of surrogate data by randomly permutating the retained time series after excluding the state stays. This analysis demonstrated that the transitions between states were not evenly distributed and a few state switches occurred more frequently than a random level ($P < 0.05$; figs. 5C and S8, Supplemental Digital Content 3, http://links.lww.com/ALN/B887). Figure 5D graphically illustrates the significant state transitions. Although there were four states observed most consistently during maintenance period (aside from burst suppression), the two states with high prefrontal-frontal connectivity (States 5 and 6) have a higher probability of transitioning into and out of each other, as do the two states with high frontal-parietal connectivity (States 7 and 8). Interestingly, States 5 and 6, rather than States 7 and 8, switched to burst suppression with a higher probability, while State 6, which is associated with alpha prefrontal-frontal connectivity, is the most probable state when exiting burst suppression. Collectively, these results demonstrate that it is more probable to stay in a certain state than switching to another state, but when a switch occurs, particular between-state transitions are more probable than others.

Discussion

In this study, we investigated functional cortical connectivity in healthy volunteers using an anesthetic protocol that is more consistent with surgical anesthesia than typical experimental protocols in healthy volunteers. Furthermore, without the varying stimulus levels of surgery, we were able to observe intrinsic brain state changes during prolonged and pharmacologically stable periods of general anesthesia. The analysis demonstrated a shifting trajectory of dominant states during anesthesia-induced alterations of consciousness. The cortical connectivity was dynamic during the isoflurane maintenance period, which cannot be explained by the small variations in isoflurane concentrations or hemodynamic factors (figs. S9 and S10, Supplemental Digital Content 4, http://links.lww.com/ALN/B888). The transition analysis revealed that the cortical connectivity was more likely to stay in a certain state than switching to another state, but when switches occurred, specific between-state transitions occurred more frequently than chance. In other words, these transitions were structured and suggest the possibility of metastability in brain activity during general anesthesia.[13]

These findings have clinical implications for future neuromonitoring strategies. Cortical connectivity remains dynamic during general anesthesia and no one-dimensional oscillatory or connectivity pattern appears to reliably distinguish levels of consciousness. For example, past studies have demonstrated a consistent disruption of frontal-parietal connectivity after induction of general anesthesia,[11] as well as anteriorized and hypercoherent alpha oscillations associated with propofol and halogenated ethers.[9] However, the current study demonstrated that States 7 and 8, characterized by high frontal-parietal connectivity and low prefrontal-frontal connectivity, could reemerge and be persistent during the isoflurane maintenance period (figs. 2 and 4D). This suggests that neither frontal-parietal connectivity nor increased prefrontal alpha connectivity alone may serve as reliable discriminators for differentiating conscious states. Given that the spectral properties of States 7 and 8 were not specific in the alpha (8 to 13 Hz) band as for the baseline-dominant State 1, the spectral dynamics of frontal-parietal and prefrontal-frontal patterns could be more informative for the differentiation of unconscious from conscious states. Furthermore, multidimensional, network-based measures may be required for designing future neuromonitoring strategies.[23] In particular, measures that incorporate network topology, connectivity, and information integration

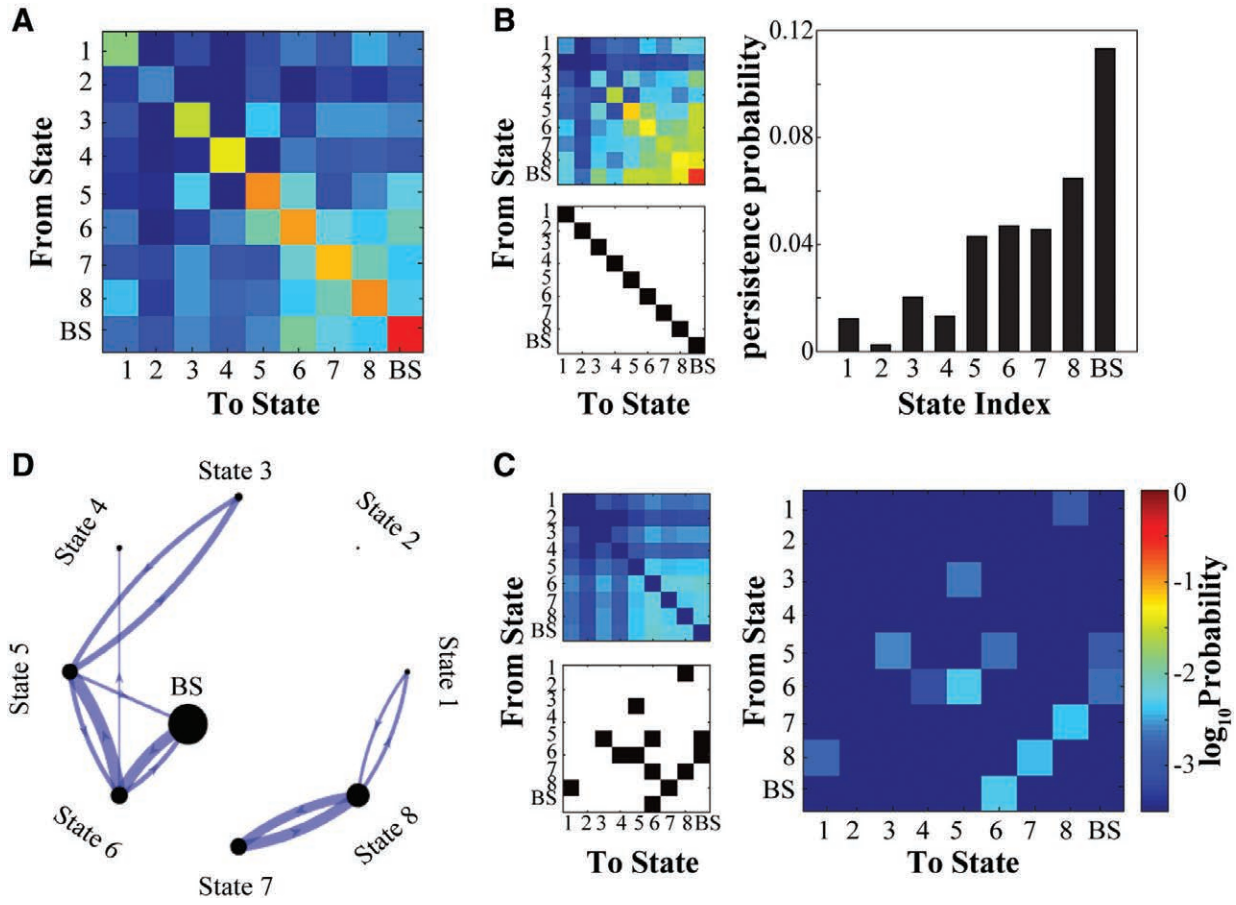

Fig. 5. Connectivity state transitions during the isoflurane maintenance period. (*A*) the transition probability matrix, with each off-diagonal element indicating the probability of switching from any state in a given row to another state in the given column, while the element on the diagonal line indicating the probability of staying in a certain state. (*B*) Relative to random transitions, cortical connectivity is more probable to stay in a certain state. (*Top left*) The mean transition probability matrix from N = 1,000 surrogate data generated by permutating the temporal order while keeping the occurrence rates of states. (*Bottom left*) The cells in *black* indicated that for all the states, the persistence probability in the state is higher than random transitioning (false discovery rate [FDR]-adjusted $P < 0.05$). (*Right*) The significant persistence probabilities in each state after subtracting the mean of those from surrogate data. (*C*) A few between-state transitions are more probable and not random. (*Top left*) The mean between-state transition probabilities from N = 1,000 surrogate data generated by randomly permutating the retained time series after removing the state stays. (*Bottom left*) The cells in *black* indicated that the transition probability is higher than random level (FDR-adjusted $P < 0.05$). (*Right*) The significant between-state transition probabilities after subtracting the mean of those from surrogate data. (*D*) Graphical representation of significant state transitions. Each node indicates a state, the size of the node is proportional to the persistence probability in each state, and the directed, weighted edges are proportional to the transition probability between the two states. BS, burst suppression.

have shown promising results in the differentiation of anesthetic states.[23] However, these potential strategies must also be assessed in real-world perioperative settings.

Many studies of unconsciousness and anesthesia have focused on the averaged connectivity pattern by assuming temporal invariance over data epochs spanning several minutes.[29,31–33] Although these studies provide valuable information in terms of identifying stereotypical signatures associated with unconsciousness induced by diverse anesthetics, they are not sufficient to reveal the full picture of cortical connectivity, which has been shown to fluctuate over time.[12,34] To resolve the issue, a number of studies have avoided the time-averaging step and quantified the averaged time-varying profiles across the participants.[2,3,8,35] Although this approach may reveal the evolution of cortical connectivity at the group level, it could obscure the fluctuations of connectivity patterns at the individual level since they occur at different times across the participants.[13] In this study, we employed cluster analysis to investigate the time-varying changes of cortical connectivity in individual participants and then pooled the data for characterization of the temporal dynamics at a group level. As compared to the static

connectivity analysis (fig. S1, Supplemental Digital Content 1, http://links.lww.com/ALN/B885), it provided useful information of the temporal progression of cortical connectivity during alterations of consciousness induced by general anesthesia (fig. 4, B to D). We identified not only averaged connectivity changes across participants, as presented in the group-level connectogram (fig. S2, Supplemental Digital Content 1, http://links.lww.com/ALN/B885), but also the co-occurrence of multiple connectivity states across the participants (fig. 4D). Overall, the analysis revealed a considerably richer picture of the temporal dynamics of cortical connectivity during general anesthesia.

During the prolonged period of isoflurane exposure, burst suppression was present and dominant in the first hour, which became less common in the last hour (fig. 1, B and D). Accordingly, the gradual shifting from burst suppression to other connectivity states (fig. 4D) may reflect the reconfiguration of the functional brain network. Indeed, general anesthesia is posited to induce changes in network configuration as reflected by increased path length, increased clustering coefficient, reduced network efficiency, and disrupted hub structure.[10,29,36,37] Altered connectivity patterns, as demonstrated in figure 4D, may provide a window into the cortical connectivity changes that occur with the functional network reconfiguration during general anesthesia. Further investigation is required to understand how the alterations between multiple connectivity states relate to such network perturbations.

The presence of state alterations might suggest the metastability of cortical connectivity during the pharmacologically stable maintenance period (figs. 3 and 5). As noted, metastability refers to a state that falls outside the stable equilibrium but persists for an extended period of time; metastability typically arises in a self-organized dynamic system and is characterized by complex patterns of fluctuations.[38] According to the integrated information theory[39] and the dynamic core hypothesis,[40] metastability can be associated with a rich repertoire of intrinsic brain states, with the diversity of the repertoire essential to consciousness, and anesthesia may suppress consciousness by constraining this repertoire.[27,41-44] In this study, rather than the dynamic changes of the repertoire of intrinsic states from wakefulness to anesthesia, we investigated the functional configurations of cortical connectivity associated with the alterations during general anesthesia, with the dynamic connectivity assessed on a relatively slower time-scale for a reliable frequency-resolved connectivity estimation.[19] This was motivated by previous research by Hudson *et al.*,[12,13] which suggested metastability in rats during steady-state isoflurane exposure up to 1 h as assessed by spectral power of local field potentials. Our findings of dynamic cortical connectivity in humans could provide further evidence for metastability in brain activity during general anesthesia. We acknowledge the possibility that the observed state alterations could arise from other underlying neural mechanisms. For example, an array of synchronization dynamics that can emerge in neuronal ensembles (such as generalized synchronization, metastability, and multistability) has been proposed as a source of the spontaneous fluctuations in empirical observations.[45]

In this study of dynamic cortical connectivity during surgical levels of anesthesia, unique methodologic strengths are worth highlighting. First, the anesthetic protocol is clinically relevant but without the confound of surgical intervention. This provides a prolonged, pharmacologically stable period that allowed us to investigate the dynamic cortical connectivity. Second, we defined the frequency-resolved frontal-parietal and prefrontal-frontal weighted phase lag index as the connectivity pattern, which enabled us to characterize the temporal, spatial, and spectral dynamics of cortical connectivity associated with anesthesia-induced alterations of consciousness. Third, a few practical strategies in implementation include integrating the time windows with burst suppression into the characterization of brain dynamics and detecting the significant state transitions by means of two successive sets of surrogate data. Collectively, this enabled the characterization of the complex dynamics of cortical connectivity in humans during general anesthesia.

There were also methodologic limitations of the study that should be considered. First, we investigated the complex spectral dynamics with a good spectral resolution while smearing the temporal resolution, which is constrained by the unfortunate tradeoff between resolution in time and frequency. Second, the spatial discretization of scalp electroencephalographic recordings is limited. We only focused on the spatial connectivity properties among a few regions determined by our prior findings. The time-varying whole brain functional connectivity patterns might be a useful direction for further studies. Third, the employed k-means clustering approach assumes that all samples can be categorized into a set of mutually exclusive clusters, which means each connectivity pattern was forcibly assigned to one of the eight clusters. A probabilistic clustering algorithm, such as a Gaussian mixture model, may improve the clustering performance by incorporating the degree of uncertainty for cluster assignment. Fourth, the state transition analysis conceptually implies a first-order Markov model of the connectivity state time series, while temporal dependencies more complex than the model cannot be captured by this approach. A systematic analysis of the temporal properties of the connectivity state sequences is an area for future studies.

In summary, we demonstrated that functional connectivity, as assessed by weighted phase lag index, is spatial-spectral specific and dynamic despite a stable surgical level of isoflurane anesthesia. The complex spectral dynamics of frontal-parietal and prefrontal-frontal connectivity provide insight in the neurobiology of general anesthesia and inform the practical use of connectivity for monitoring brain states.

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Competing Interests

The authors declare no competing interests.

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