Rapid fragmentation of neuronal networks at the onset of propofol-induced unconsciousness

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AUTHOR SUMMARY

General anesthesia involves rapidly inducing a reversible coma by administering a large dose of a fast-acting drug, such as propofol. Previous research has demonstrated that propofol enhances inhibitory input to neurons throughout the spinal cord, brainstem, thalamus, and cortex (1). However, how these effects in single cells translate to largescale neural circuits and cause unconsciousness is not well understood. We recorded spiking activity from ensembles of single neurons and intracranial electrical activity during the induction of propofol general anesthesia in human subjects undergoing surgery. We found that loss of consciousness (LOC) corresponds to the abrupt onset of a slow cortical oscillation that marks a fragmentation of neuronal networks. These results identify the slow oscillation as a dramatic neural correlate of LOC and demonstrate that slow oscillations mark the transition into a brain state in which local neuronal networks are isolated, impairing both temporal and spatial communication throughout the cortex.

How general anesthetic drugs produce unconsciousness remains an open question in neuroscience. One current hypothesis is that unconsciousness is the result of a breakdown of information integration in the cortex (2, 3). However, the mechanism by which this breakdown might occur is not known. Most studies have used deeply anesthetized animals to probe this question, but they were unable to identify the neural changes corresponding to LOC because of the difficulty of continuously monitoring animal consciousness. To address this question, we measured neuronal and network-level electrophysiological signals in three human subjects during planned neurosurgery to remove electrodes implanted to diagnose medically refractory epilepsy. These recordings included intracranial electrocorticograms spanning up to 8 cm of the cortex, local field potentials, and ensembles of single neurons (n = 198). The subjects received propofol and were instructed to perform a simple task in which they responded to sounds by pressing a button; the 5-s window in which they stopped performing the task was defined as the time of LOC.

We first examined spike rates in single neurons to test whether propofol’s inhibitory effects caused widespread suppression of neuronal activity. We found that spike rates dropped significantly after LOC, but this decrease occurred 0–30 s after LOC, rather than simultaneously with the change in behavioral state. In addition, we observed large fluctuations in spike rates during the unconscious period that varied between 30% and 115% of the baseline rate during the conscious state. Therefore we concluded that unconsciousness is not strictly associated with a reduction in mean neuronal activity.

Given that neuronal activity was not necessarily suppressed during unconsciousness, we next examined whether a change in the network structure of spiking occurred at LOC. We found that LOC occurred simultaneously with the onset of a slow (<1 Hz) oscillation in the local field.

![Propofol-induced loss of consciousness occurs at the onset of a slow oscillation that is associated with fragmented neuronal networks. (A) Loss of consciousness occurs simultaneously with the onset of the slow oscillation. Unconsciousness is not associated with consistent changes in mean spike rates; instead, spiking is grouped into short windows locked to the trough of the slow (~1 Hz) oscillation. These troughs occur at different times in different cortical regions, thereby fragmenting activity in different brain regions into distinct, asynchronous windows. LFP, local field potential. (B) Slow oscillation marks a state in which functional connectivity can be preserved within small (~4 mm) neuronal networks; however, processing within a local network is interrupted periodically by suppression of spiking. Global communication between brain regions also is impaired, because these suppressions occur at different times across cortical areas.](http://www.pnas.org/cgi/doi/10.1073/pnas.1210907109)
potential. Changes in other frequency bands also occurred, including a decrease in theta (3–8 Hz power) and increases in alpha (∼10 Hz) and gamma (25–40 Hz) power (4), but these features fluctuated after LOC, most likely in relation to propofol drug level. In contrast, slow oscillation power increased abruptly at LOC and remained elevated throughout the recording. Previous studies in deeply anesthetized animals have shown that this oscillation is associated with neurons alternating between UP (activated) and DOWN (inactivated) states (5). The extracellular recordings collected here demonstrate an analogous alternation between ON (activated) and OFF (inactivated) states during unconsciousness. We found that this alternation occurred in nearly all single units and developed within seconds of LOC. Neuronal processing therefore was limited to intervals of a few hundred milliseconds, which were interrupted periodically by suppression lasting several hundred milliseconds.

We next analyzed how these periods of suppression were distributed across the brain. We analyzed the slow oscillation phase and found that distant cortical areas frequently were at different phases. Because the phase was strongly associated with periods of suppressed activity, this finding implied that different brain regions were active at different times. These asynchronous periods of suppression therefore would be expected to impair global cortical communication, because active neurons in one area would be unable to propagate information effectively to a distant, inactivated brain region.

Given that neuronal communication was impaired over large timescales and across distant cortical regions, we next investigated whether connectivity in local (<4 mm) networks was impaired. Unexpectedly, we found that millisecond-scale connectivity was preserved during general anesthesia, suggesting that relationships between nearby neurons were not disrupted substantially during unconsciousness. In addition, we found that the history of ensemble unit spiking was useful for predicting future spiking, indicating that significant structure remained in the units during unconsciousness. These results suggest that significant aspects of local functional connectivity were preserved during general anesthesia, although for short timescales. However, neuronal activity was fragmented into brief temporal windows, thereby preventing both temporal and spatial information processing.

We conclude that although propofol produces a broad range of oscillatory and neuronal dynamics, slow oscillation is correlated most specifically with LOC. We show that slow oscillation marks a fragmented brain state that impairs communication between distant cortical areas and over long timescales, despite preserved structure in small neuronal networks (Fig. P1). Because slow oscillations can be observed in normal sleep, certain types of coma, and complex-partial seizures, it is possible that specific types of slow oscillation dynamics are associated with different arousal states. One limitation of our study is that the subjects had epilepsy; the consistency of our results across subjects suggests that their underlying pathology did not affect their neural response to propofol, but future experiments could investigate this question further in patients with different pathologies. Future studies also could investigate the relationship between specific types of slow oscillations and behavioral states and test whether they can be used clinically to monitor patient consciousness during surgical procedures. Our results are consistent with the hypothesis that unconsciousness during general anesthesia is caused by a breakdown of cortical integration (2, 3) and suggest a mechanism for how propofol could induce this state.

Supporting Information

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SI Methods

Spectrograms were calculated with sliding windows of 5 s and three tapers computed every 1 s for a time-bandwidth product of 2 and spectral resolution of 0.4 Hz. Bandpower measures were computed with smoother taper settings to collapse across frequencies (10 s every 5 s, 39 tapers, time-bandwidth product of 20, and spectral resolution of 2 Hz) and then were median-filtered across five time bins to remove brief transient effects caused by low-frequency artifacts. Bandpower plots (Fig. 2B and Fig. S1 A–D) show the mean power spectral density (averaged across frequencies within a band). Triggered spectrograms were calculated with a window of 0.5 s every 0.125 s, using three tapers for a time-bandwidth product of 2 and spectral resolution of 4 Hz. Pre- and post-loss of consciousness (LOC) spectra were computed across 90 s with nine tapers for a resolution of 0.056 Hz, and error bars (marked by shaded region) were taken from the Chronux theoretical computation method at \( P = 0.05 \).

For generalized linear model fitting, spike rates were modeled as:

\[
\lambda(t) = \frac{\exp(X)}{1 + \exp(X)} \quad \text{with} \quad X = \sum_{i=1}^{b} \beta_H^{i} N_{t-i} + \sum_{j=1}^{10} \beta_P^{j} p_{t}^{j} + \beta_A a_t
\]

where \( \lambda(t) \) is the spike rate of the ensemble at a given time \( t \), \( N_{t-i} \) is the ensemble spike history at time \( t-i \), \( p_{t}^{j} \) is 1 if the phase of the slow oscillation at time \( t \) is in bin \( j \) and 0 otherwise, and \( a_t \) is the amplitude of the local field potential (LFP) slow oscillation at time \( t \). The parameters of the generalized linear model (GLM) are \( \beta_H^{i} \) for spike history in each time bin, \( \beta_P^{j} \) for the phase bins, and \( \beta_A \) for the amplitude.
Fig. S1. Bandpower changes relative to LOC. Power in different frequency bands from a representative microelectrode LFP for each patient. None of the bands shows a strong change at LOC that is maintained throughout the post-LOC period. The dashed line indicates LOC, and arrows show times of propofol delivery (±20 s). (A) Theta power transiently decreases after LOC. (B) Alpha power transiently decreases after LOC. (C) Low gamma power is variable across patients and tends to increase minutes after LOC. (D) High gamma power is variable across patients. (E) Overall spectrum averaged across the grid of electrocorticogram (ECoG) electrodes from all patients. The blue line shows spectrum in the 90 s before LOC; the green line shows spectrum in the 90 s after LOC. The unconscious state shows an increase in both low-frequency (0.1–4 Hz) and gamma (25–50 Hz) power.
Spikes become phase-coupled to the slow oscillation at LOC, shown in patients A and C. The dashed line indicates LOC in all panels. (A and C) LFP from a representative microelectrode in patient A (panel A) and in patient C (panel C). Filtered slow oscillation is overlaid in red, and the histogram of spikes from all units is in black, showing onset of slow oscillation at LOC. The LOC period is shaded in light green and the post-LOC period in darker green. (B and D) (Left) Phase-coupling of all single units in patient A to their local LFP slow oscillation, where color indicates the percent of spikes in a given phase bin. The plot demonstrates that phase-coupling begins at LOC. (Right) The red line shows a sinusoid to indicate the relationship between phase and slow oscillation shape. The histogram shows the phase distribution of all post-LOC spikes, which are coupled to the rising phase of the slow oscillation.
Fig. S3. Slow oscillations in distant ECoG channels have variable phase offsets. The phase-locking factor (PLF) characterizes the stability of the phase offset between two oscillations over a period. The PLF magnitude ranges between 0 and 1, where 1 reflects constant phase offset, and 0 represents variable phase offset. The PLF angle indicates the average phase offset. (A and C) PLF magnitude between every electrode pair during the pre-LOC and post-LOC recording periods for patients A and B, plotted according to the distance between the electrodes in each pair. The PLF magnitude decreases significantly with distance, reflecting higher variability in phase offsets between distant ECoG electrodes. The red lines show mean (± SD) of the PLF magnitude at all electrode pairs at that distance. (B and D) 2D histogram of the PLF angle between all ECoG pairs, in the pre- and post-LOC periods, showing that the mean phase offset is more variable between distant channels, with values as large as π, than between nearby channels.
Fig. S4. After LOC, slow oscillations are asynchronous across cortex and are associated with ON/OFF states; therefore, distant cortical areas frequently are at a suppressed phase during local ON periods. (A) Position of ECoG and microelectrode recordings in patient A. Each white circle marks the location of an ECoG electrode, and the microelectrode where spikes were recorded is marked with a star. (B) Magnitude of the PLF between every ECoG electrode relative to the ECoG closest to the spike recordings (gr42). The PLF drops with distance in both the pre- and post-LOC states, showing that distant areas have variable phase offsets relative to the local recording. (C) Modulation index (MI) quantifying the strength of the spike–phase relationship. The MI is consistently low in the pre-LOC state, demonstrating the absence of a strong spike–phase relationship. After LOC, the MI is high only in local ECoG recordings, demonstrating that spikes are strongly phase-coupled to local slow oscillations and that this relationship weakens with distance.
Fig. S5. Phase-locking across the ECoG grid. Star indicates location of spike recordings. Color of circles indicates the phase of the slow oscillation at each site that is associated with maximal local spiking, and the size of the circle shows the MI at that electrode. Stars next to channel labels indicate that spiking at the microelectrode array is significantly modulated relative to the slow oscillation phase from that ECoG recording site.
Fig. S6. GLM fitting for each patient. (A–C) Akaike's Information Criterion (AIC) and Bayesian Information Criterion (BIC) for the GLM of each patient, with history ranging from 0–100 bins of 12 ms each. Number of history bins with the lowest BIC was used to construct a GLM and in each patient included least 30 ms of spike history. (D–F) Goodness-of-fit analysis. Time-rescaling plots of the best-fit GLM for each patient. A perfect model would create a uniform distribution and would be plotted as a straight line from zero to one. Dashed lines indicate 95% confidence intervals on the correct rescaling. Each plot shows that the GLM approximates the correct rate, although it does not remain within the 95% confidence bounds for a perfect rate estimate.
Contribution of each covariate to predicting spikes in the pre-LOC state

Fig. S7. GLM predicting spikes in the pre-LOC period. Parameter estimates from the best GLM for population spiking. For each patient, the best model includes information from both LFP phase and population spike history. As in the post-LOC case, recent spike history is a good predictor of future spiking, and more distant spike history contributes less. The contribution of the slow oscillation LFP phase is much smaller in the pre-LOC period than in the post-LOC period.

Fig. S8. ON period-locked effects in the ECoG channel near the spike recordings. (A) Triggered spectrogram locked to the onset of ON periods, averaged across patients A and B, with power normalized within each frequency band and log-transformed. There is increased high-frequency power during spiking activity. (B) ON period-triggered ECoG recording. The ON periods begin just before the slow oscillation trough and are not associated with an asymmetrical peak after spiking.
Table S1. Time at which each feature becomes different from baseline

<table>
<thead>
<tr>
<th>Patient</th>
<th>Spike rate decrease, s</th>
<th>Low gamma power (25–50 Hz) increase, s</th>
<th>Slow power (0.1–1 Hz) increase, s</th>
<th>MI increase, s</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>10 to 1</td>
<td>10 to 15</td>
<td>5 to 10</td>
<td>−5 to 0</td>
</tr>
<tr>
<td>B</td>
<td>25 to 30</td>
<td>170 to 175</td>
<td>0 to 5</td>
<td>5 to 10</td>
</tr>
<tr>
<td>C</td>
<td>0 to 5</td>
<td>20 to 205</td>
<td>−5 to 0</td>
<td>0 to 5</td>
</tr>
</tbody>
</table>

Time is relative to LOC and is calculated in 5-s bins. Only the slow oscillation increases reliably within ~5 s of LOC, occurring within a 5-s bin that either overlaps with LOC or is adjacent to the bin containing LOC. The MI, measuring coupling between spikes and the slow oscillation phase, also increases within a 5-s bin of LOC. Spike rates and gamma power are affected later, and the timing of those changes is more variable across patients.