Figures and figure supplements

Transcranial direct current stimulation modulates primate brain dynamics across states of consciousness

Guylaine Hoffner and Pablo Castro *et al*.

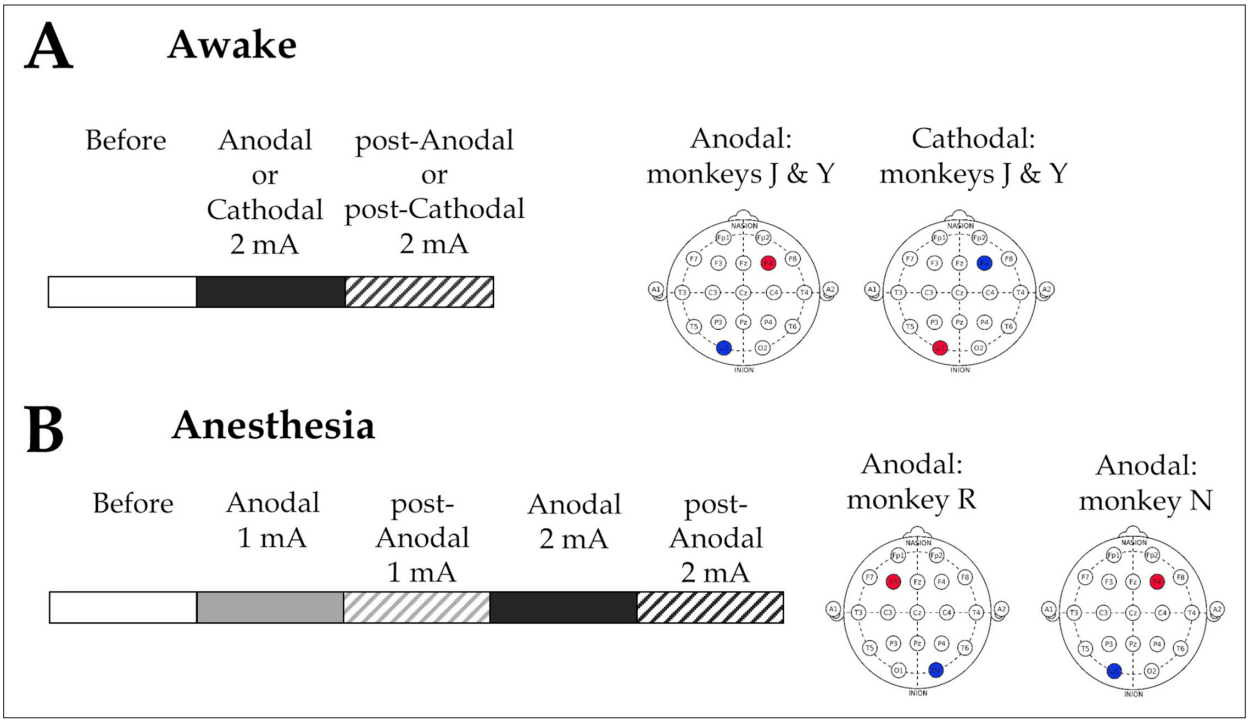

Figure 1. Schematic representation of the experimental designs and transcranial direct current stimulation (tDCS) electrode montages employed in the study. (**A**) Awake experiments. fMRI data were acquired before, during, and after 20 min of tDCS stimulation at 2 mA intensity. The anodal electrode (red) was either placed over the right prefrontal cortex (PFC; F4) and the cathodal electrode (blue) over the left occipital cortex (O1) ('anodal' montage), or a 'reversed'montage was used, with the cathodal electrode over F4 and the anodal electrode over O1 ('cathodal' montage). (**B**) Anesthesia experiments. fMRI data were recorded before, during, and after 20 min of consecutive 1 and 2 mA tDCS stimulation. An anodal electrode montage was employed, targeting the left or right PFC (F4/O1 or F3/O2) depending on the animal and its anatomical constraints. Before: before stimulation. Anodal and post-Anodal: during and after anodal tDCS of the PFC. Cathodal and post-Cathodal: during and after cathodal tDCS of the PFC.

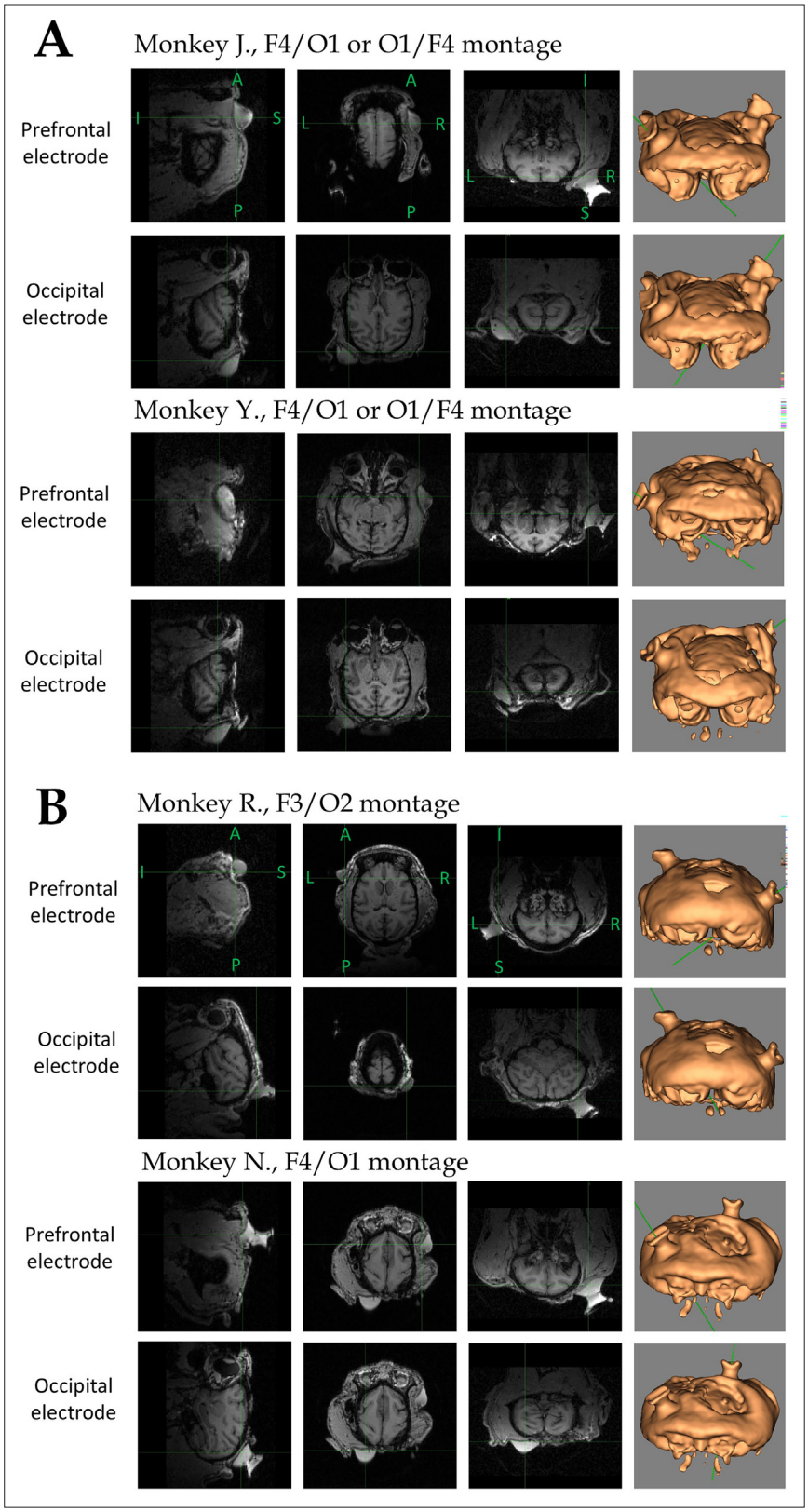

Figure 1—figure supplement 1. Structural images displaying electrode placements on the head of monkeys. (**A**) Awake experiments. Representative sagittal, coronal, and transverse MRI sections, and the corresponding skin reconstruction images showing the position of the prefrontal and the occipital electrodes on the head of monkeys J. and Y. (**B**) Anesthesia experiments. Representative sagittal, coronal, and transverse MRI sections, and the

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corresponding skin reconstruction images showing the position of the prefrontal and occipital electrodes over the occipital cortex on the head of monkeys R. and N.

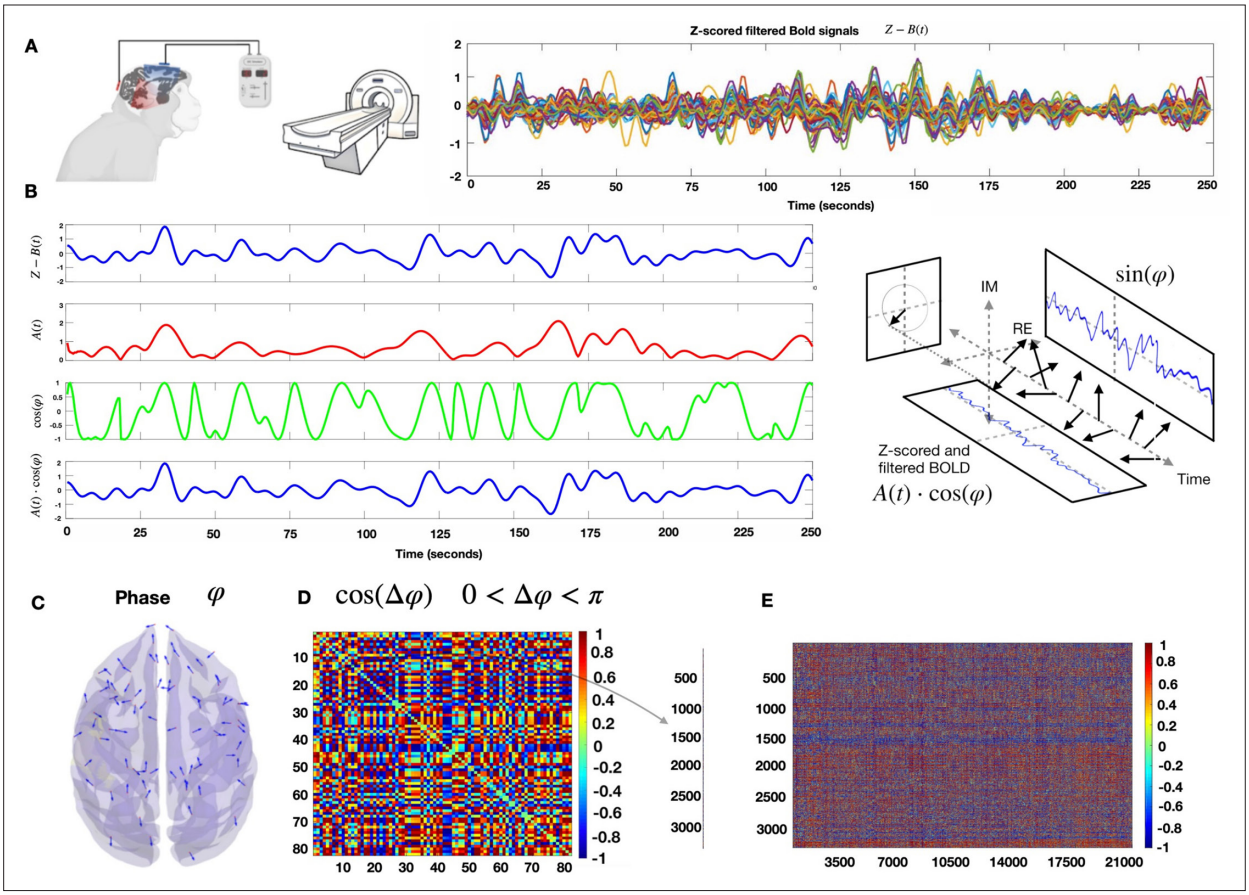

Figure 2. fMRI phase coherence analysis. (**A**) Left: Animals were scanned before, during, and after prefrontal cortex (PFC) transcranial direct current stimulation (tDCS) in the awake state (two macaques) or under deep propofol anesthesia (two macaques). Right: Example of *z*-scored filtered BOLD time series for one macaque. (**B**) Hilbert transform of the *z*-scored BOLD signal of one region of interest (ROI) into its time-varying amplitude $A(t)$ (red) and the real part of the phase φ (green). In blue, we recover the original *z*-scored BOLD signal as $A(t)\cos(\varphi)$. (**C**) Example of the phase of the Hilbert transform for each brain region at one TR. (**D**) Symmetric matrix of cosines of the phase differences between all pairs of brain regions. (**E**) We extract the superior triangular half of the phase coherence matrix and vectorize it. All time points of the recordings from the different conditions are concatenated together.

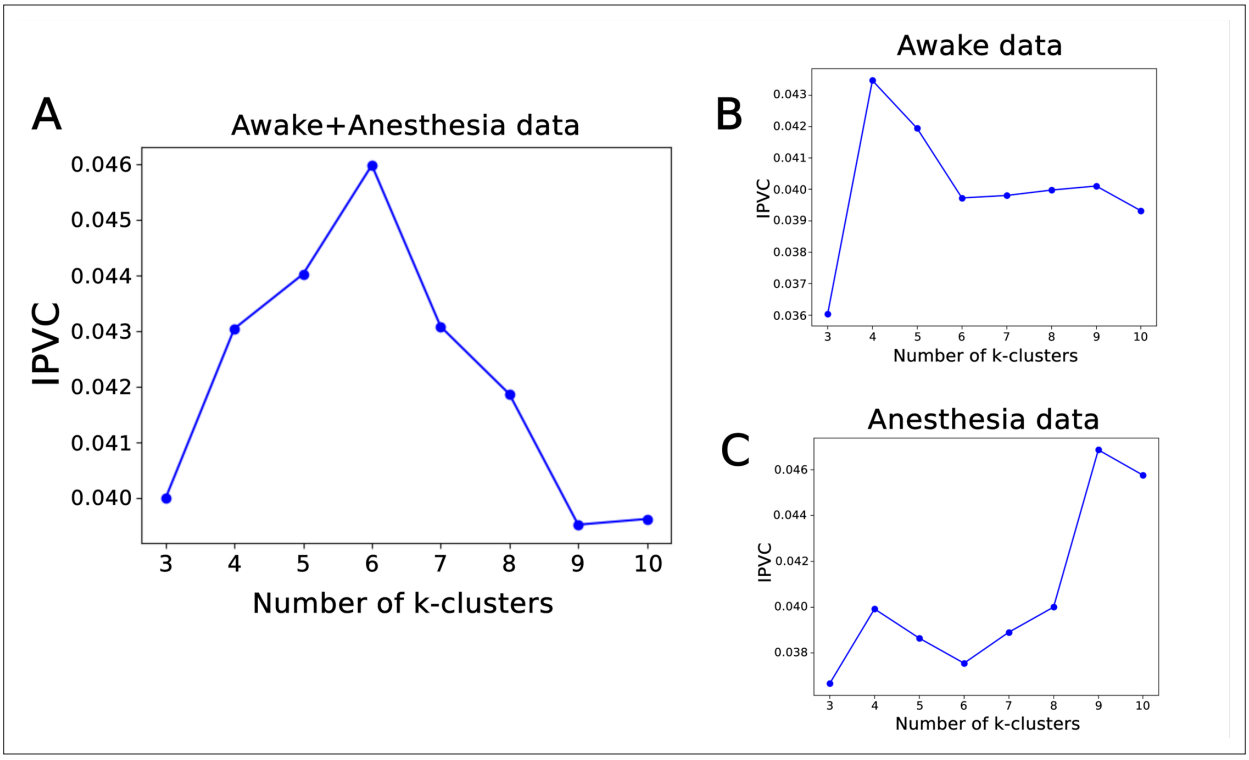

Figure 2—figure supplement 1. Inter-pattern correlation variance (IPCV), a measure used to identify the optimal number of *k* clusters (or brain patterns). (**A**) IPCV obtained when awake and anesthesia datasets are considered together. (**B**) IPCV obtained when only the awake dataset is considered. (**C**) IPCV obtained with only the anesthesia dataset.

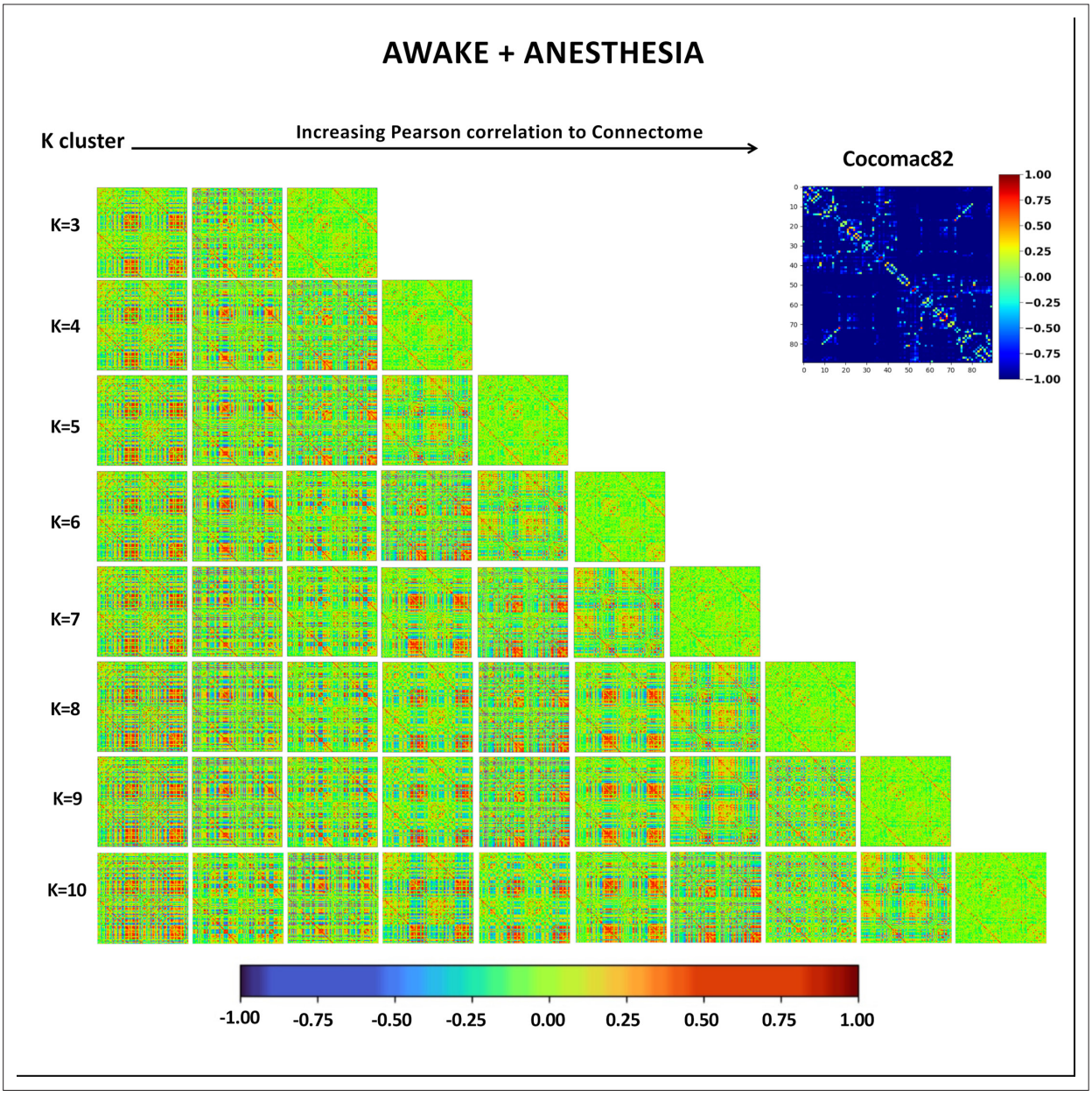

Figure 2—figure supplement 2. Dynamical functional patterns from awake and anesthesia datasets. Matrix representation of the brain patterns for *k* = 3–10 number of clusters in the *k*-means algorithm. The brain patterns were obtained by including all the awake (five conditions) and anesthesia (five conditions) data in the analysis and ordered from least (left) to most (right) similarity to the structural connectivity matrix.

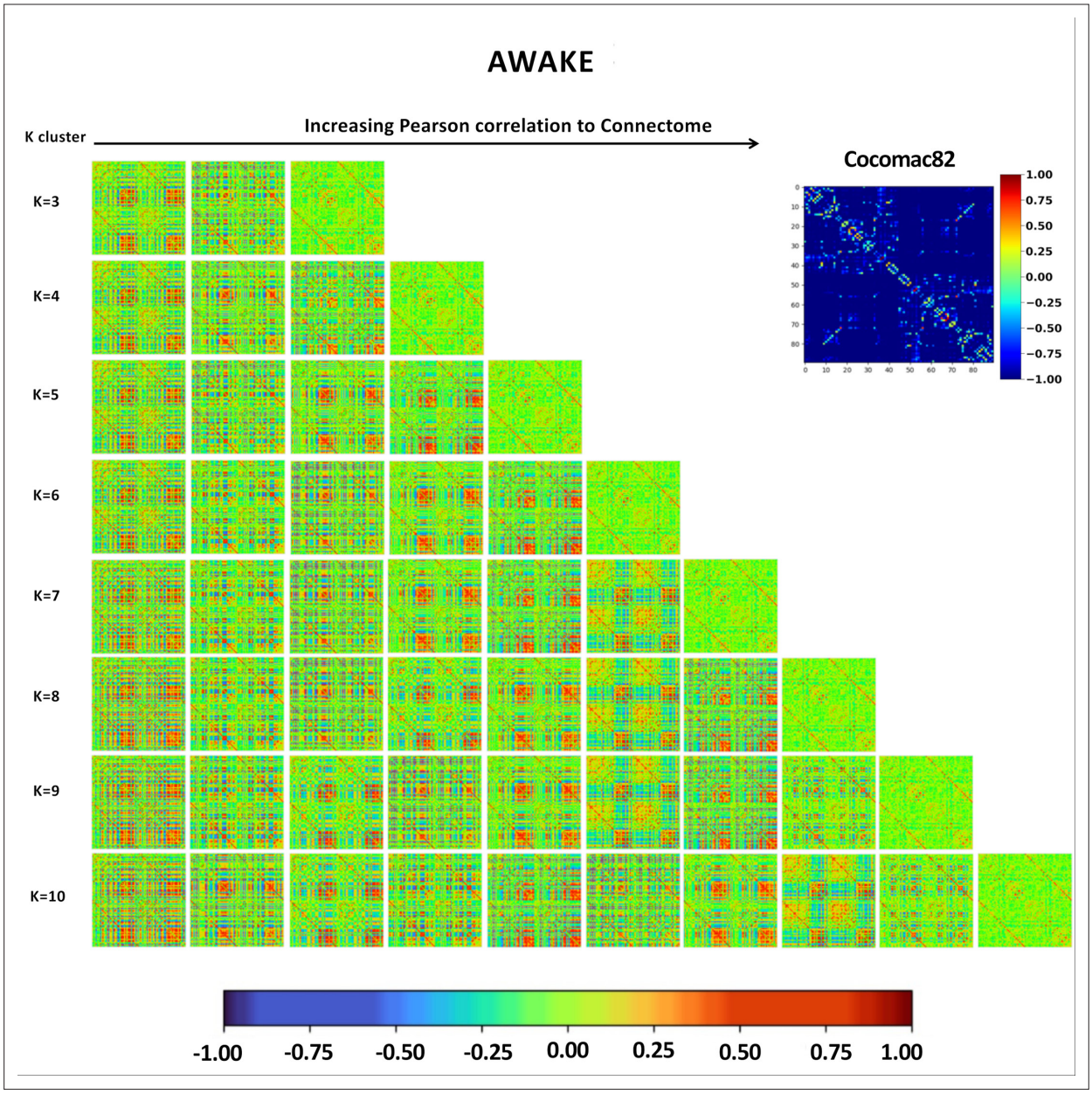

Figure 2—figure supplement 3. Dynamical functional patterns from the awake dataset. Matrix representation of the brain patterns for k = 3–10 number of clusters in the k-means algorithm. The brain patterns were obtained by including all five awake conditions and were ordered from least (left) to most (right) similarity to the structural connectivity matrix.

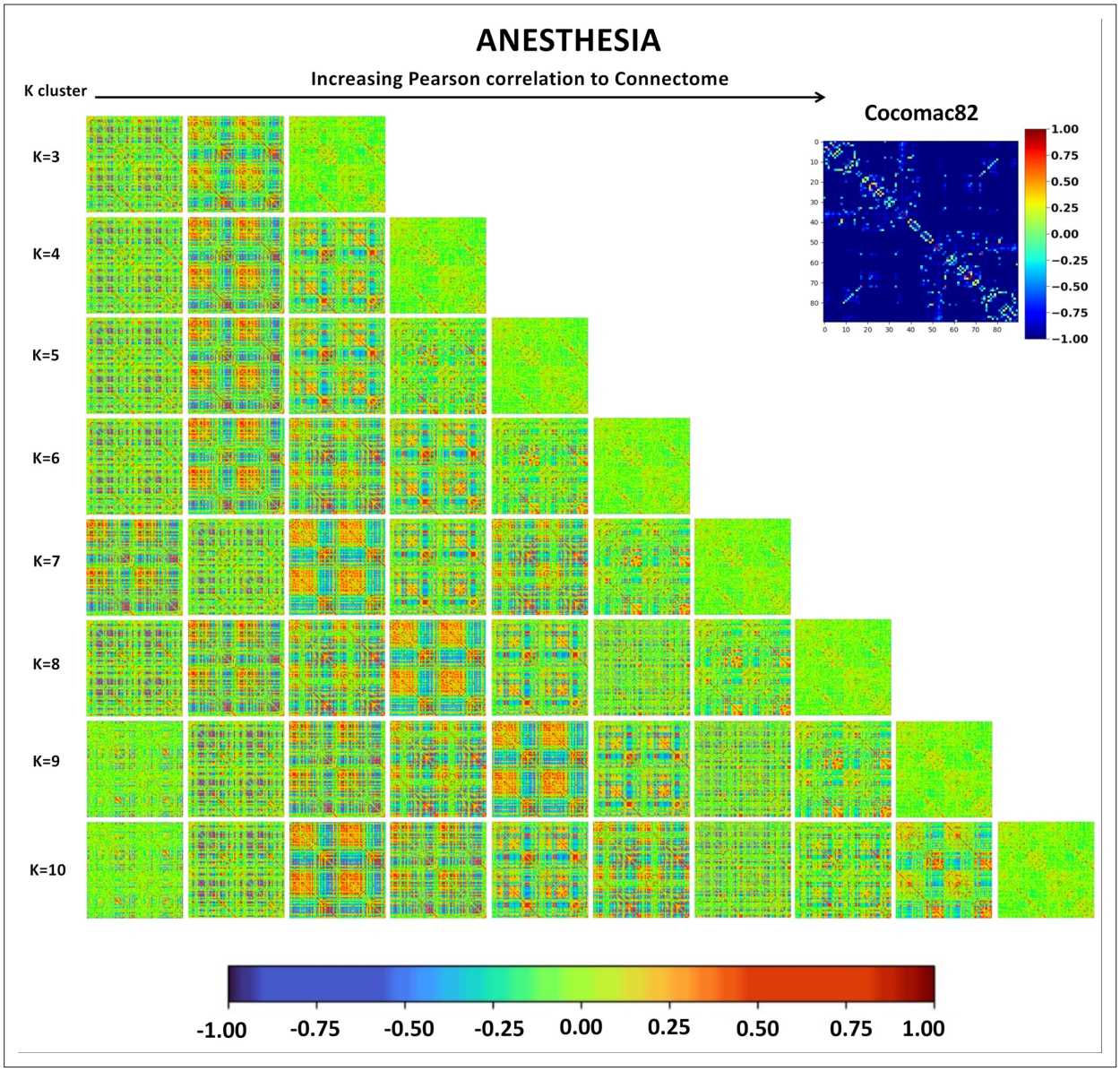

Figure 2—figure supplement 4. Dynamical functional patterns from the anesthesia dataset. Matrix representation of the brain patterns for k = 3–10 number of clusters in the k-means algorithm. The brain patterns were obtained by including all five anesthesia conditions and were ordered from least (left) to most (right) similarity to the structural connectivity matrix.

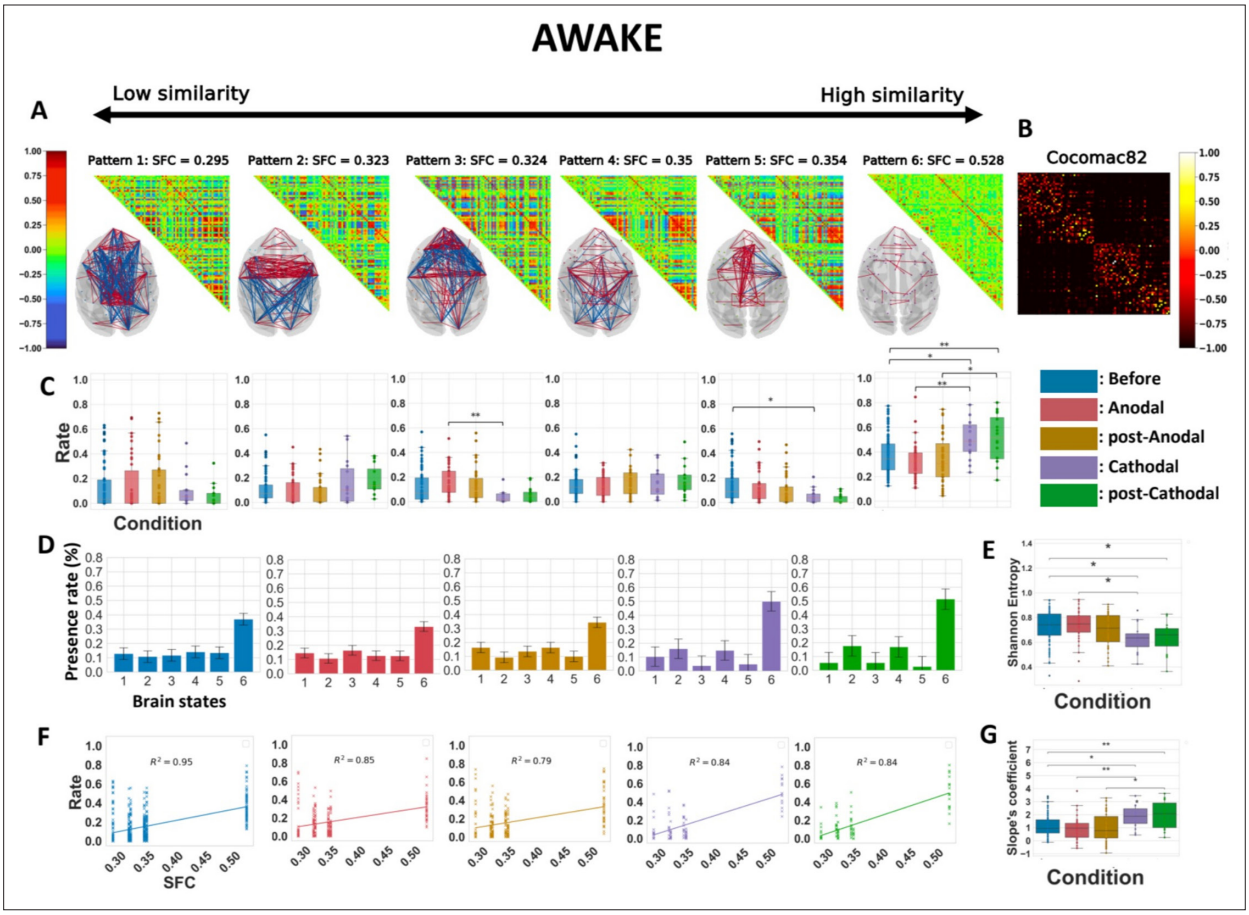

Figure 3. Cathodal but not anodal transcranial direct current stimulation (tDCS) of the prefrontal cortex (PFC) alters the repertoire of functional brain patterns, increases structure–function coupling, and decreases Shannon entropy in awake animals. (**A**) Matrix representation of the brain patterns for $k = 6$, obtained by including all the five awake conditions. Brain coordination patterns are ranked from least (left) to highest (right) correlation to the structural connectivity matrix. Networks are plotted in anatomical space (transverse view), showing only values <−0.5 and >0.5. Red lines represent positive synchronizations between regions of interest, and blue lines represent negative ones. (**B**) Macaque connectome in the CoCoMac82 parcellation. (**C**) Rate of occurrence of each brain connectivity pattern across the five conditions. Boxplots show median occurrence rates with interquartile range and maximum–minimum values (whiskers). (**D**) Barplots of the six brain states and their average presence rate over the different conditions. (**E**) Normalized Shannon entropy as a function of the conditions. (**F**) Rates of occurrence of brain patterns as a function of their similarity in functional and structural connectivity (SFC) for the five conditions. Lines are calculated, per conditions, based on the best linear fit between the average presence rate of each pattern and their SFC. R^2, Spearman correlation. Crosses represent individuals' presence rates. (**G**) Coefficient of the linearly regressed slope across conditions. Before: pre-stimulation. Anodal and post-Anodal: during and after anodal tDCS of the PFC (F4/O1 montage). Cathodal and post-Cathodal: during and after cathodal tDCS of the PFC (O1/F4 montage). Asterisks indicate statistically significant differences between conditions (*: $p < 0.05$; **: $p < 0.01$).

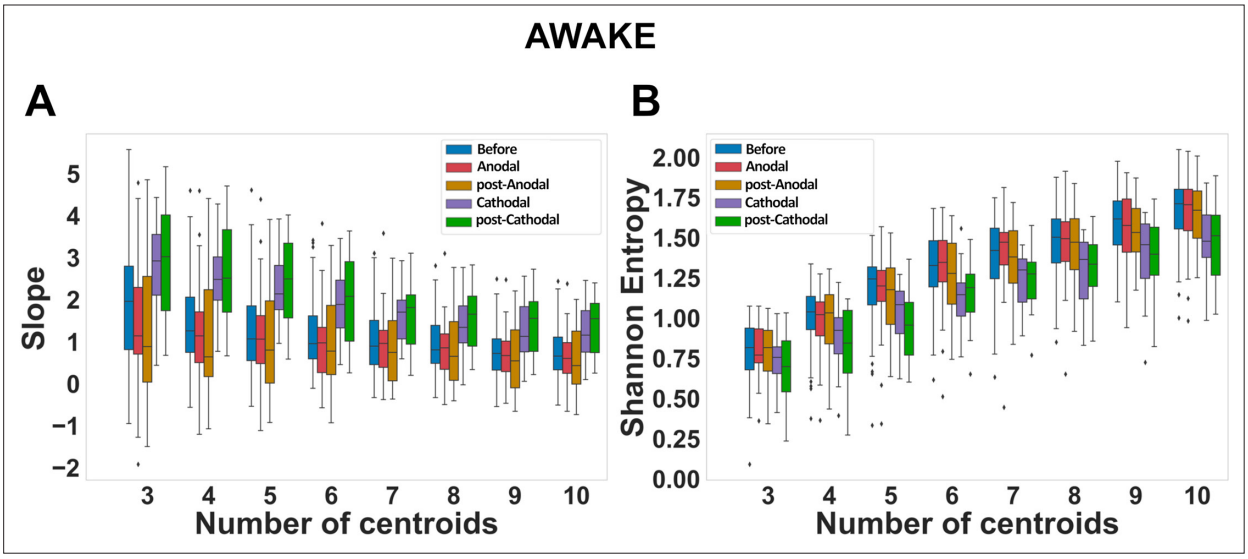

Figure 3—figure supplement 1. Slope and Shannon entropy in the awake conditions. Slopes of the linear regression (**A**) and Shannon entropy (**B**) of the distribution of brain pattern occurrences computed for a number of clusters from 3 to 10 in the *k*-means algorithm. All five awake conditions were included in the analyses. The same relative differences were found for all values of *k* analyzed. In the cathodal prefrontal stimulation conditions, the slopes of the linear regression show a tendency toward anatomically driven dynamics, and the Shannon entropy is lower than in the pre-stimulation and anodal prefrontal stimulation conditions.

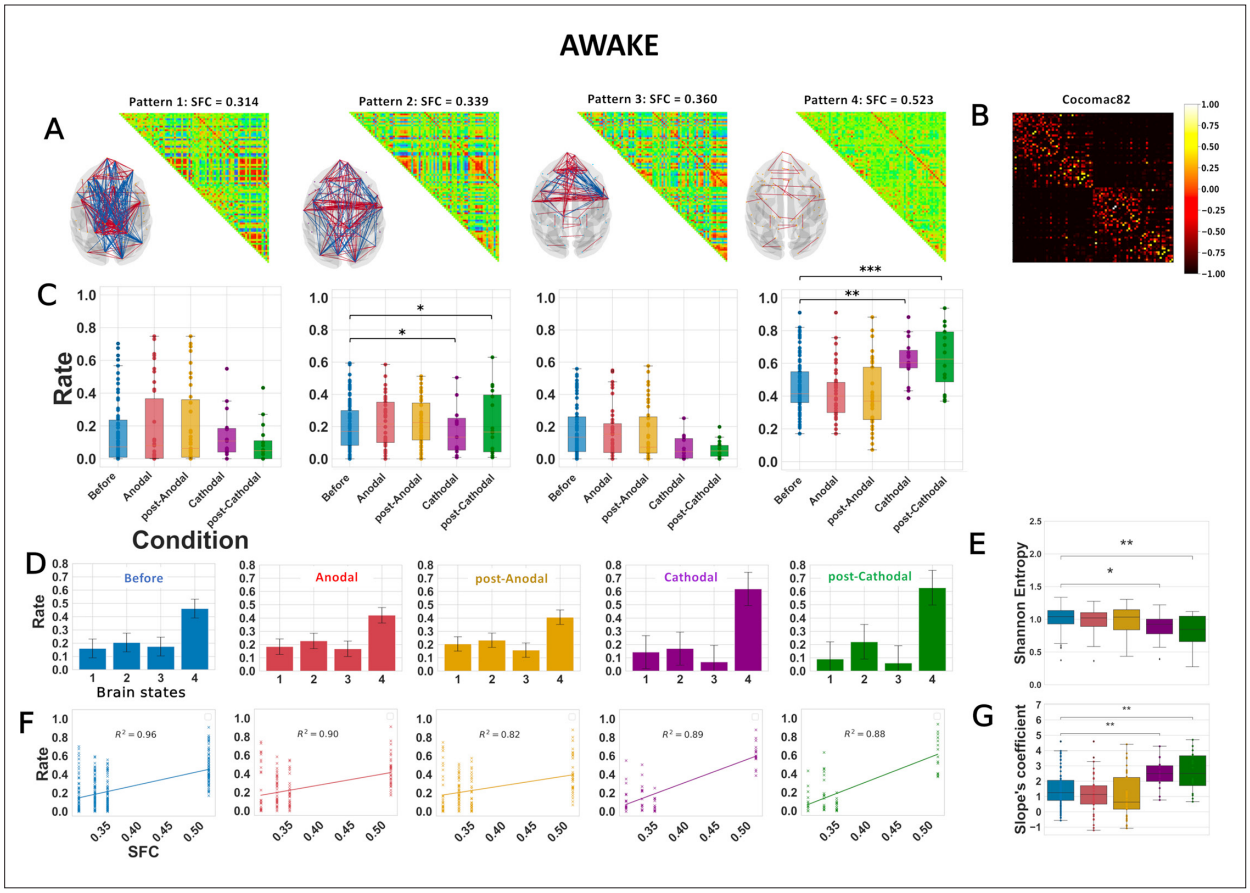

Figure 3—figure supplement 2. Dynamical functional connectivity analysis of the awake dataset with $k = 4$ numbers of clusters. (**A-G**) as in Figure 3. Similar analyses to those shown in ***Figure 3*** (panels **A–G**) are presented here for $k = 4$, considering all five awake conditions. Before: before transcranial direct current stimulation (tDCS) in the awake state. Anodal and post-Anodal: during and after anodal tDCS of the prefrontal cortex (PFC) (F4/O1 electrode montage) at an intensity of 2 mA. Cathodal and post-Cathodal: during and after cathodal tDCS of the PFC (O1/F4 montage) at an intensity of 2 mA. Asterisks indicate statistically significant differences between conditions (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).

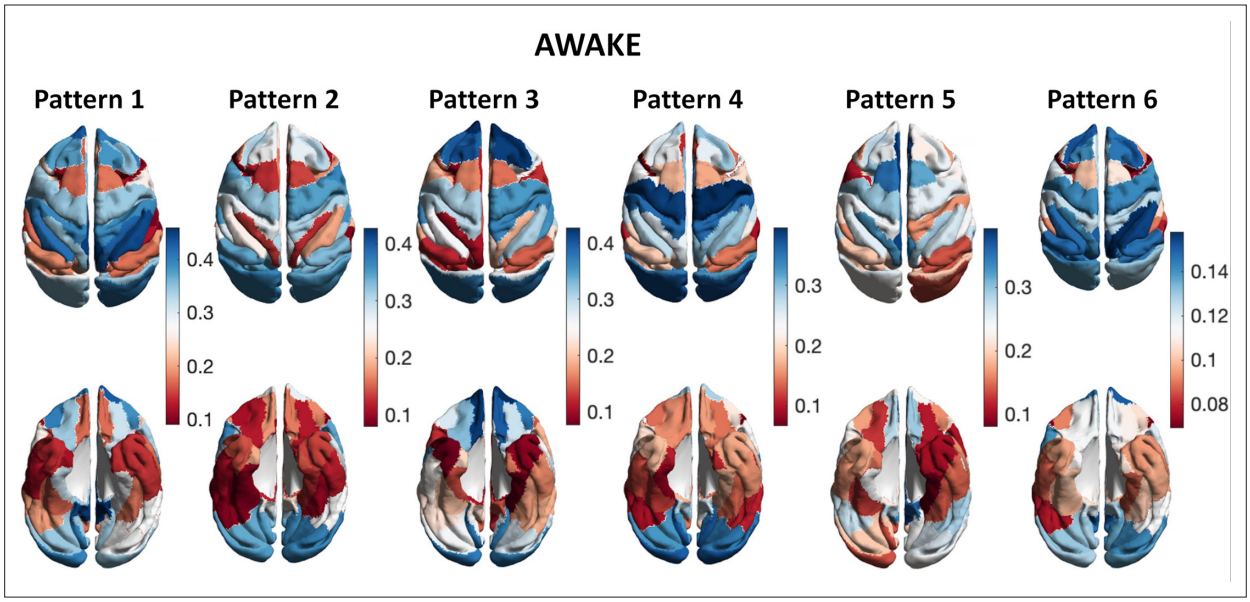

Figure 3—figure supplement 3. Variance in inter-region phase coherences of brain patterns. Low values (red and light red) indicate that the distribution of synchronizations between a brain region and the rest of the brain has relatively low variance, while high values (blue and light blue) indicate relatively high variance. Both supra (top) and subdorsal (bottom) views are displayed for each brain pattern from the main figure, ordered similarly as previously: from left (1) to right (6) as their respective structure–function correlation (SFC) increases.

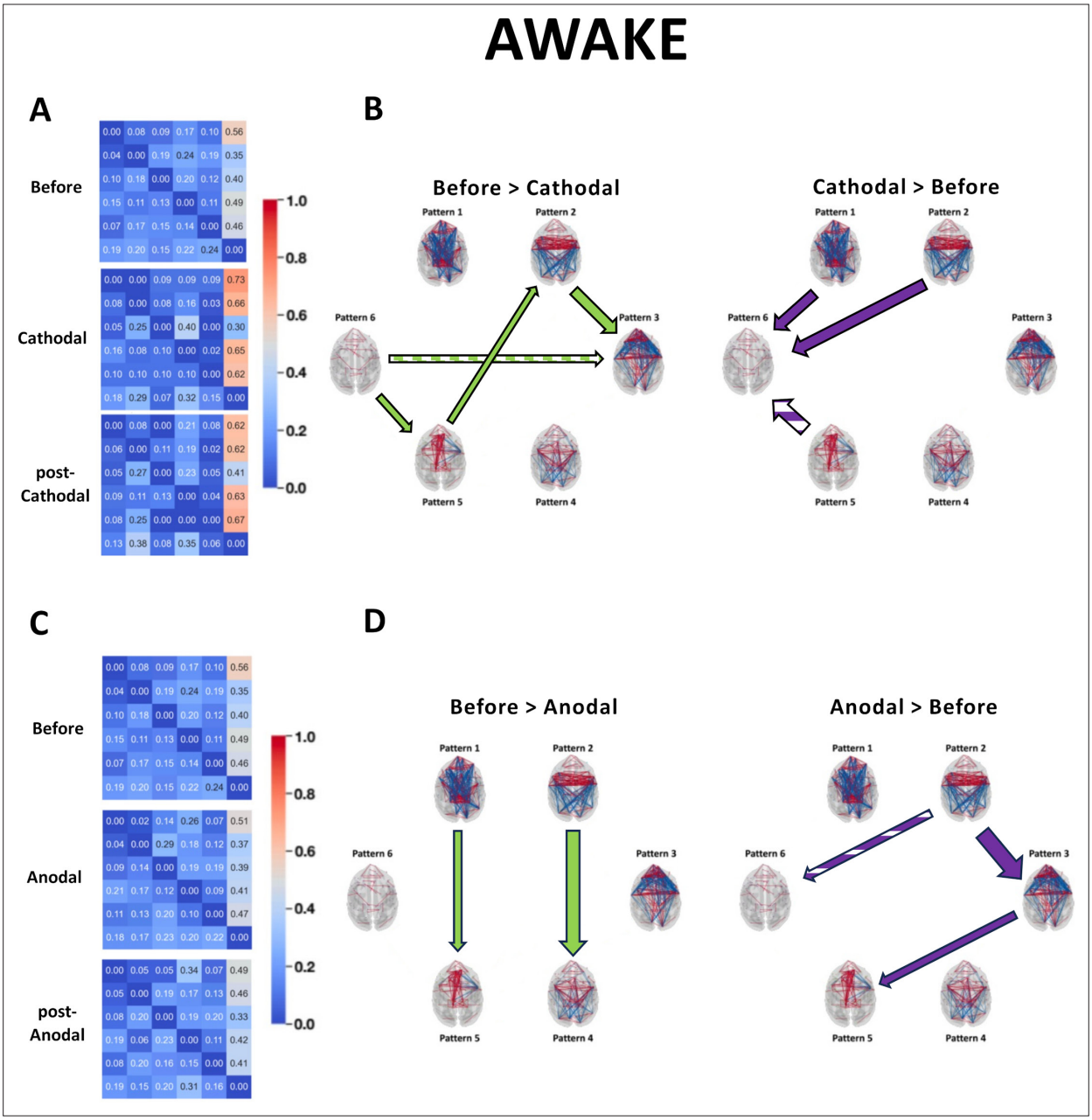

Figure 4. Transition probabilities and Markov chain analysis of the identified brain states during and after cathodal or anodal prefrontal cortex (PFC) stimulation in the awake state. Overall transition probabilities matrices pre-stimulation, during, and after cathodal stimulation (**A**) or during and after anodal stimulation (**C**). (**B**, **D**) Left panels: transition probabilities greater before stimulation than during stimulation (full green arrows) or post-stimulation (hashed green arrows). Right panels: transition probabilities greater during (full purple arrows) or after (hashed purple arrows) the stimulation than before. Arrow size indicates p-value significance. Before: pre-stimulation. Cathodal and post-Cathodal: during and after cathodal transcranial direct current stimulation (tDCS) of the PFC (O1/F4 montage). Anodal and post-Anodal: during and after anodal tDCS of the PFC (F4/O1 montage).

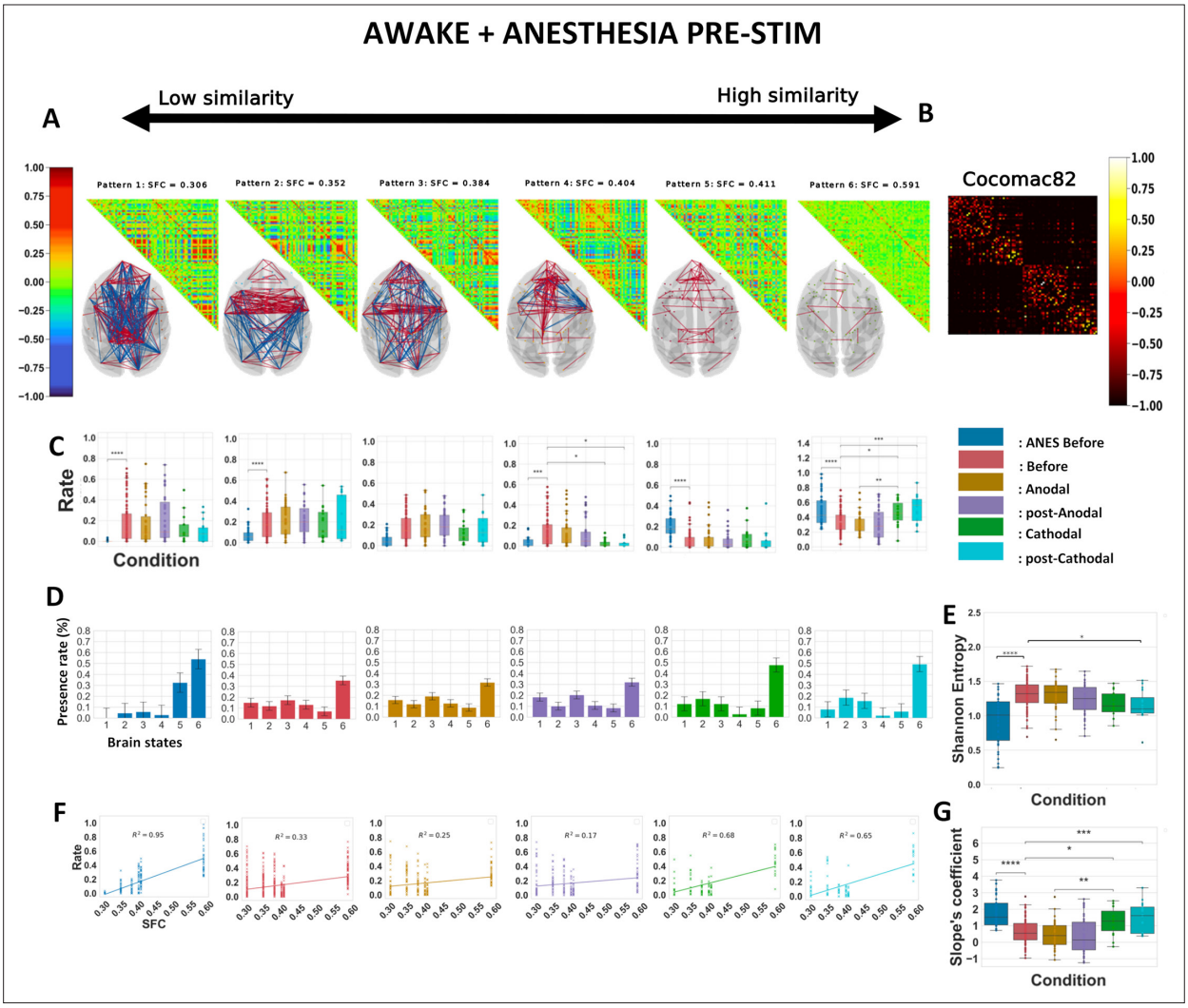

Figure 5. The polarity-dependent effects of prefrontal transcranial direct current stimulation (tDCS) on awake brain dynamics resist contrasting with anesthetized dynamics. Similar analyses to those shown in ***Figure 3*** (panels **A–G**) are presented here for $k = 6$, considering all five awake conditions as well as the anesthesia baseline condition. ANES before: before stimulation under anesthesia. Before: pre-stimulation. Anodal and post-Anodal: during and after anodal tDCS of the PFC (F4/O1 montage). Cathodal and post-Cathodal: during and after cathodal tDCS of the PFC (O1/F4 montage). Similar analysis. Asterisks indicate statistically significant differences between conditions (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ****: $p < 0.0001$).

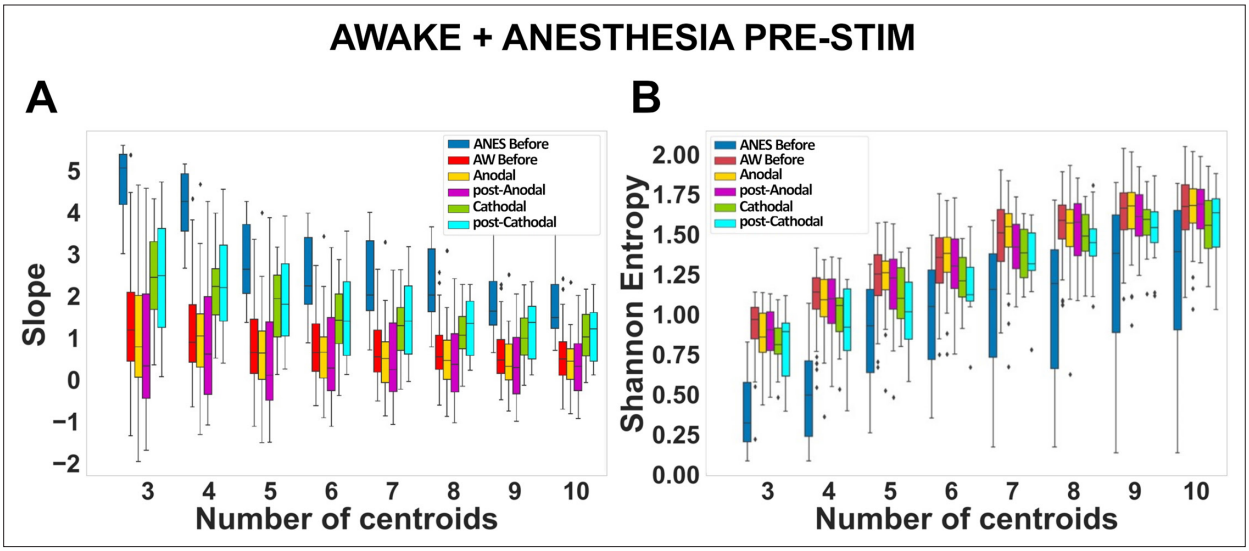

Figure 5—figure supplement 1. Slope and Shannon entropy in awake conditions contrasted with anesthesia pre-stimulation condition. Slopes of the linear regression (left panel) and Shannon entropy (right panel) of the distribution of brain pattern occurrences computed for a number of clusters from 3 to 10 in the *k*-means algorithm. All five awake conditions plus the anesthesia pre-stimulation condition were included in the analyses. The same relative differences were found for all values of *k* analyzed. In the cathodal prefrontal stimulation conditions, the slopes of the linear regression are increased and tend to approach the values of the anesthesia pre-stimulation condition. Similarly, the Shannon entropy is lower in the cathodal stimulation conditions than in the pre-stimulation and anodal prefrontal stimulation conditions and moves toward the value of the anesthesia pre-stimulation condition.

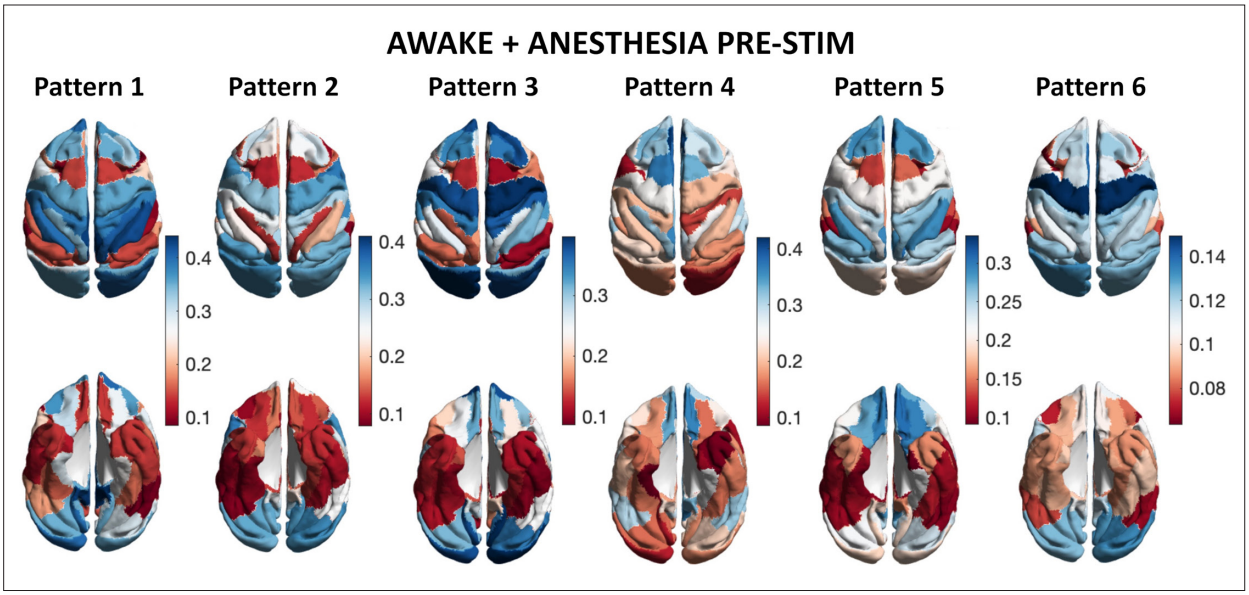

Figure 5—figure supplement 2. Variance in inter-region phase coherences of brain patterns. Low values (red and light red) indicate that the distribution of synchronizations between a brain region and the rest of the brain has relatively low variance, while high values (blue and light blue) indicate relatively high variance. Both supra (top) and subdorsal (bottom) views are displayed for each brain pattern from the main figure, ordered similarly as previously: from left (1) to right (6) as their respective structure–function correlation (SFC) increases.

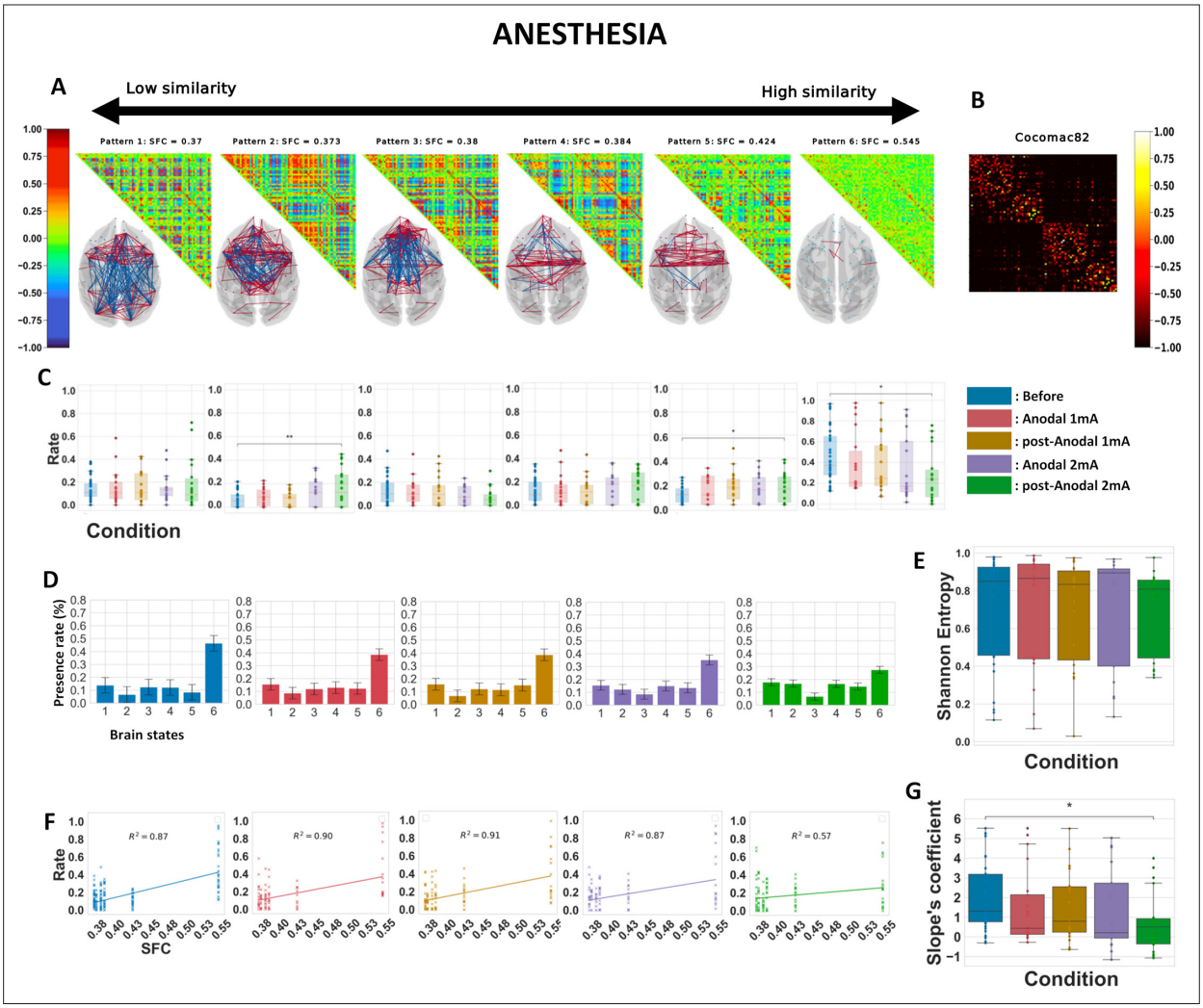

Figure 6. Anodal 2 mA transcranial direct current stimulation (tDCS) of the prefrontal cortex (PFC) modifies cortical brain dynamics and reduces structure–function coupling in anesthetized animals. Similar analyses to those in ***Figures 3 and 5*** (panels **A–G**) are presented here for $k = 6$, considering all the five anesthesia conditions. Before: before stimulation. Anodal 1 mA and post-Anodal 1 mA: during and after anodal tDCS of the PFC (F4/O1 or F3/O2) delivered at an intensity of 1 mA. Anodal 2 mA and post-Anodal 2 mA: during and after anodal tDCS of the PFC (F4/O1 or F3/O2 montage) delivered at an intensity of 2 mA. Asterisks indicate statistically significant differences between conditions (*: $p < 0.05$; **: $p < 0.01$).

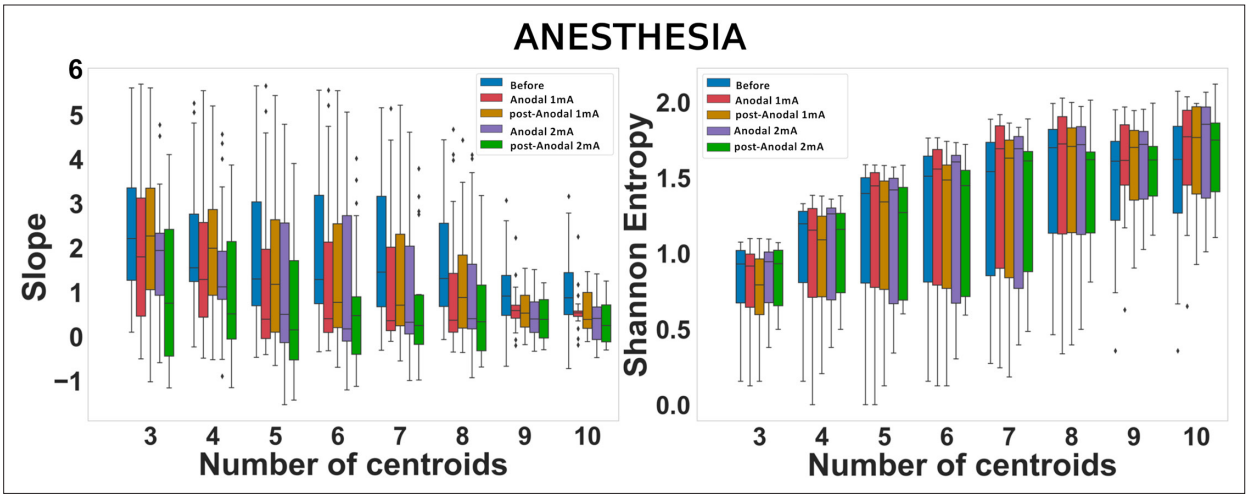

Figure 6—figure supplement 1. Slope and Shannon entropy in anesthesia conditions. Slopes of the linear regression (left panel) and Shannon entropy (right panel) of the distribution of brain patterns occurrences computed for a number of clusters from 3 to 10 in the *k*-means algorithm. All five anesthesia conditions were included in the analyses. The same relative differences were found for all values of *k* analyzed. In the 2 mA stimulation conditions, the slopes of the linear regression are decreased compared to the pre-stimulation condition.

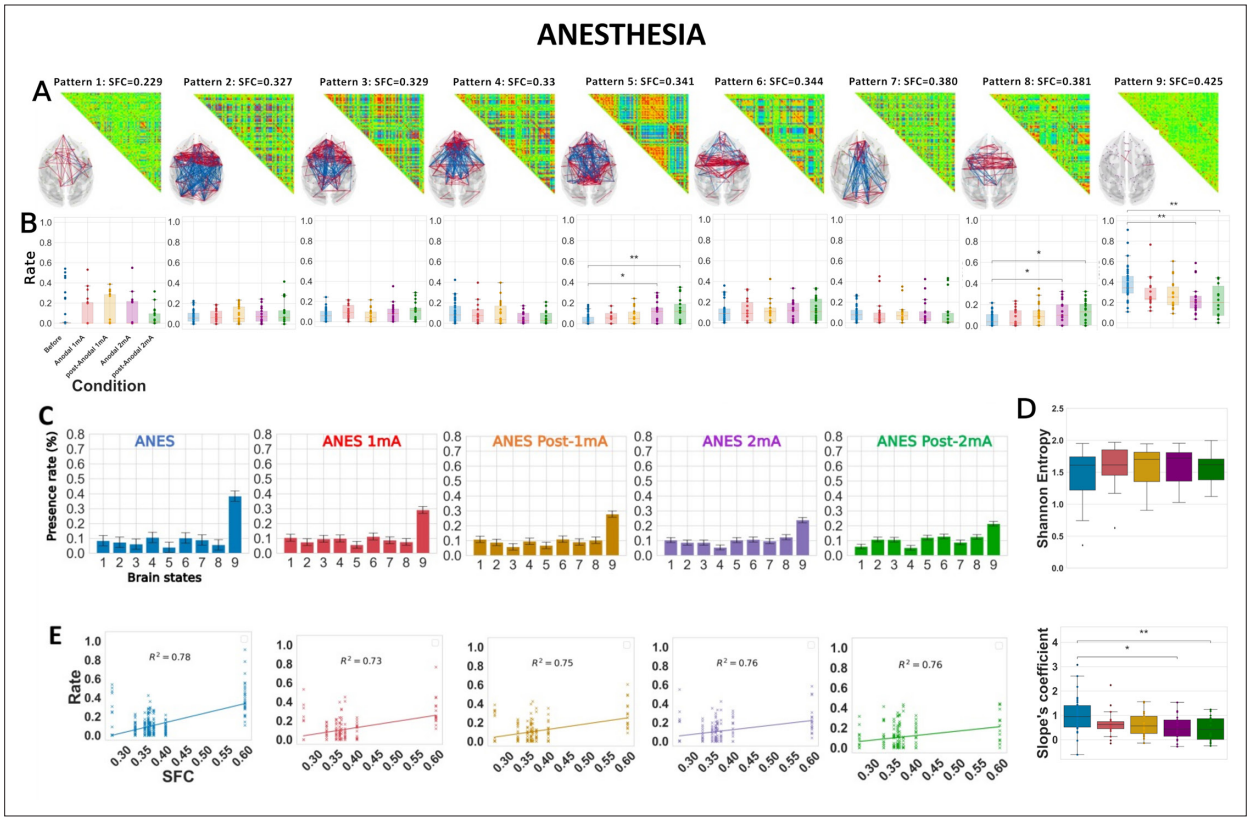

Figure 6—figure supplement 2. Dynamical functional connectivity analysis of the anesthesia dataset with $k = 9$ numbers of clusters. Similar analyses to those in ***Figure 6*** (panels **A-E**) are presented here for $k = 9$, considering all the five anesthesia conditions. Before: before stimulation, under anesthesia. Anodal 1 mA, post-Anodal 1 mA: during and after anodal transcranial direct current stimulation (tDCS) of the prefrontal cortex (PFC) at 1 mA intensity. Anodal 2 mA and post-Anodal 2 mA: during and after anodal tDCS of the PFC at 2 mA intensity. Asterisks indicate statistically significant differences between conditions (*: $p < 0.05$; **: $p < 0.01$).

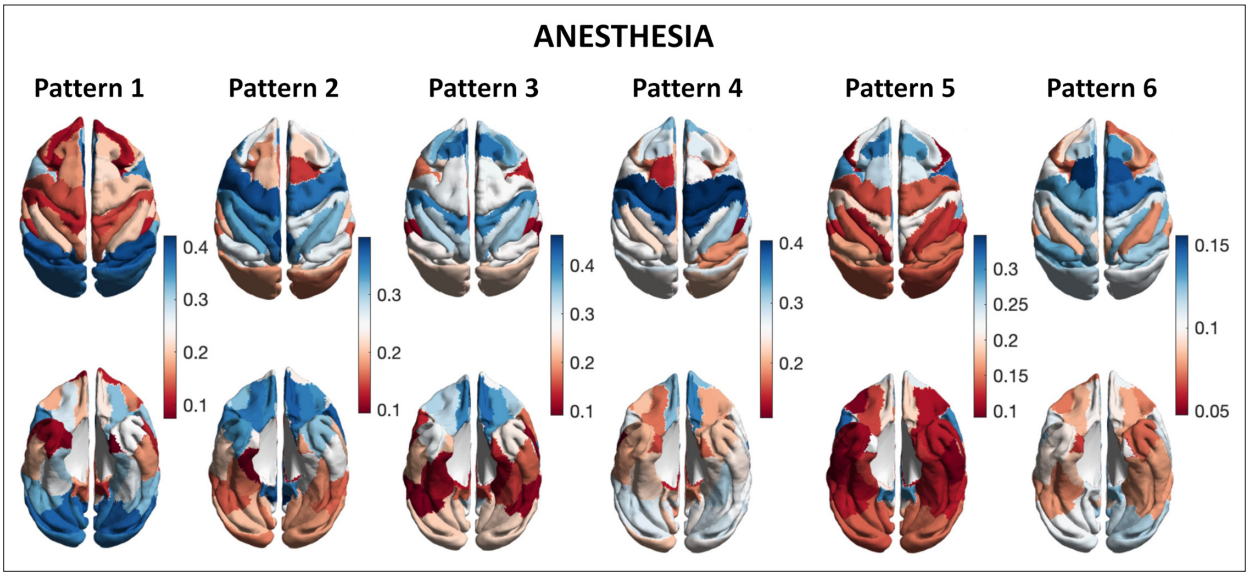

Figure 6—figure supplement 3. Variance in inter-region phase coherences of brain patterns. Low values (red and light red) indicate that the distribution of synchronizations between a brain region and the rest of the brain has relatively low variance, while high values (blue and light blue) indicate relatively high variance. Both supra (top) and subdorsal (bottom) views are displayed for each brain pattern from the main figure, ordered similarly as previously: from left (1) to right (6) as their respective structure–function correlation (SFC) increases.

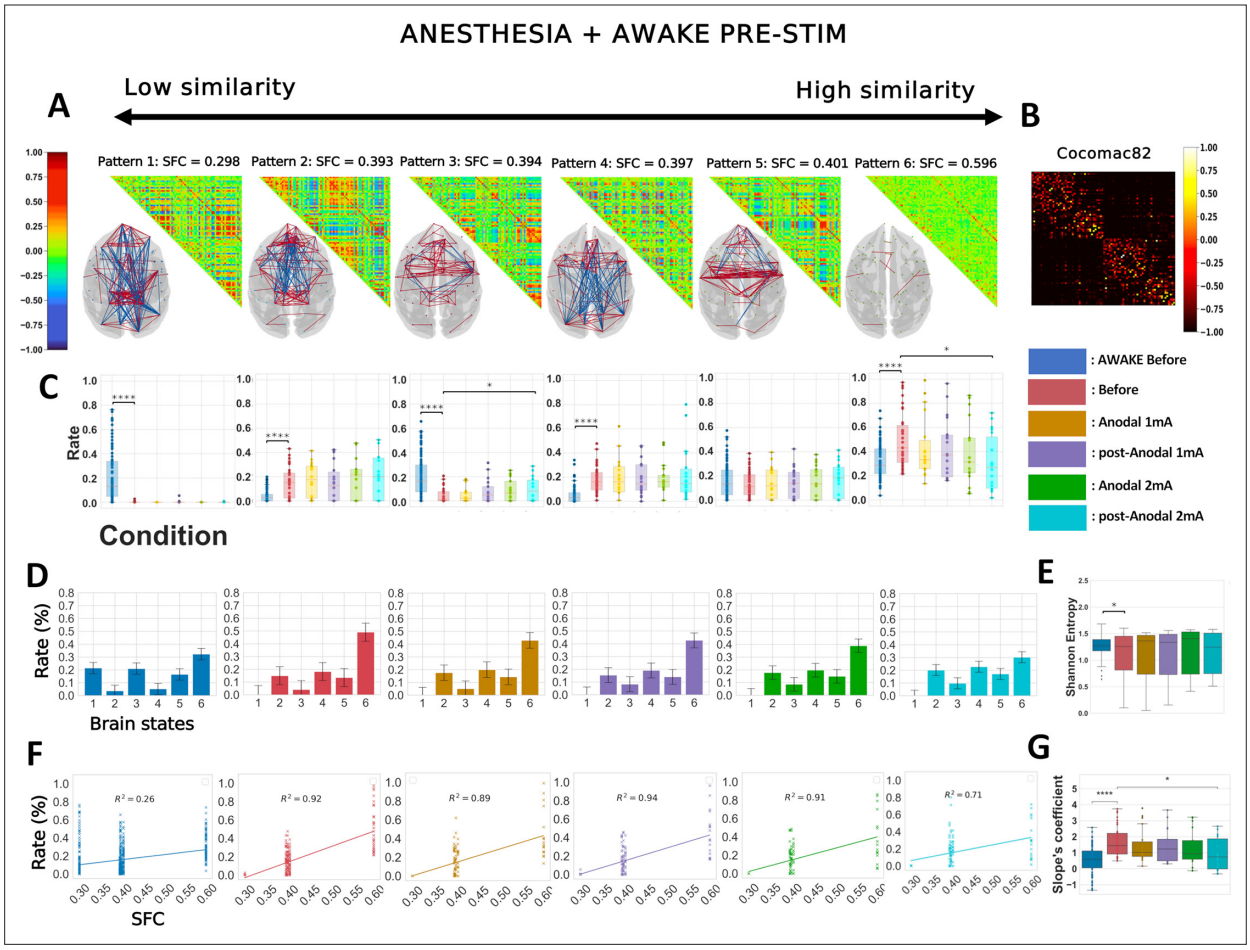

Figure 7. The effects of 2 mA anodal prefrontal transcranial direct current stimulation (tDCS) on anesthesia brain dynamics resist contrasting with awake dynamics. Similar analyses to those in ***Figure 6*** (panels **A-G**) for $k = 6$ are presented here, considering all the five anesthesia conditions plus the awake pre-stimulation condition. Awake before: before stimulation in the awake state. Before: before stimulation, under anesthesia. Anodal 1 mA and post-Anodal 1 mA: during and after anodal tDCS of the prefrontal cortex (PFC) at 1 mA intensity. Anodal 2 mA and post-Anodal 2 mA: during and after anodal tDCS of the PFC at 2 mA intensity. Asterisks indicate statistically significant differences between conditions (*: $p < 0.05$; ****: $p < 0.0001$).

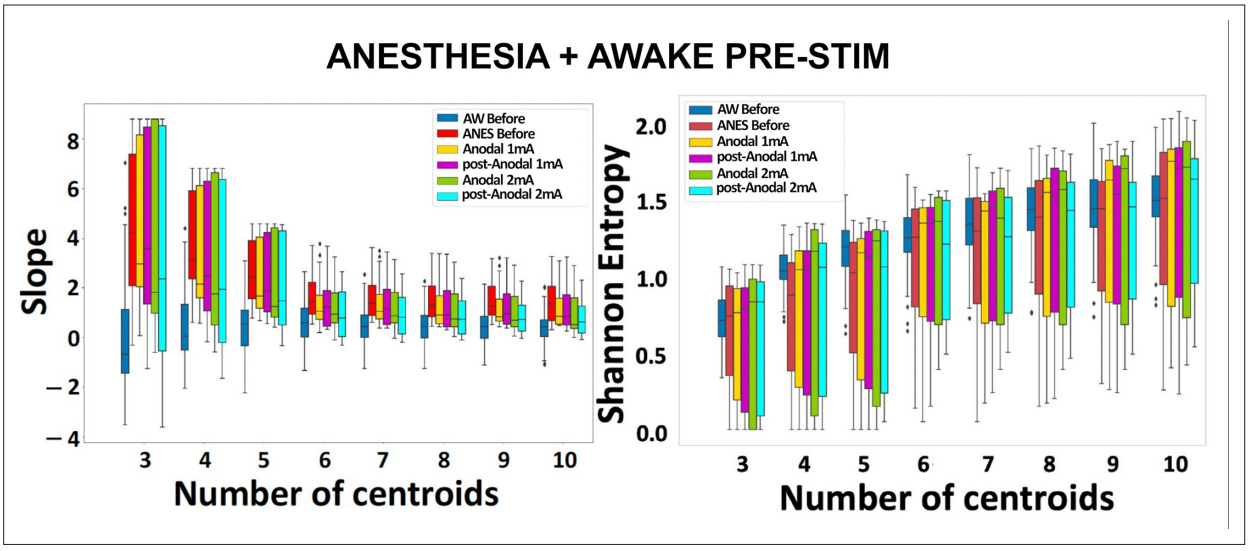

Figure 7—figure supplement 1. Slope and Shannon entropy in anesthesia conditions contrasted with awake pre-stimulation condition. Slopes of the linear regression (left panel) and Shannon entropy (right panel) of the distribution of brain pattern occurrences computed for a number of clusters from 3 to 10 in the *k*-means algorithm. All five anesthesia conditions plus the awake pre-stimulation condition were included in the analyses. The same relative differences were found for all values of *k* analyzed. In the 2 mA stimulation conditions, the slopes of the linear regression are decreased and tend to approach the values of the awake pre-stimulation condition.

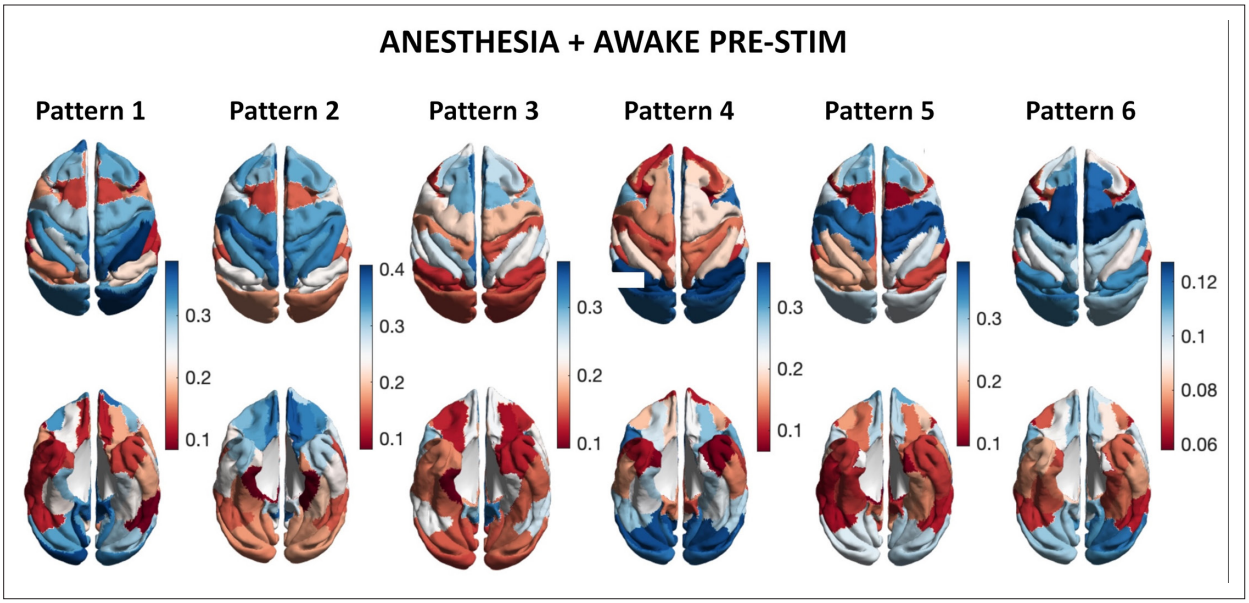

Figure 7—figure supplement 2. Variance in inter-region phase coherences of brain patterns. Low values (red and light red) indicate that the distribution of synchronizations between a brain region and the rest of the brain has relatively low variance, while high values (blue and light blue) indicate relatively high variance. Are displayed both supra (top) and subdorsal (bottom) views for each brain pattern from the main figure, ordered similarly as previously: from left (1) to right (6) as their respective structure–function correlation (SFC) increases.