Electrographic Measures of Functional Connectivity May Reflect Different Neural Processes After Fluid Percussion Injury

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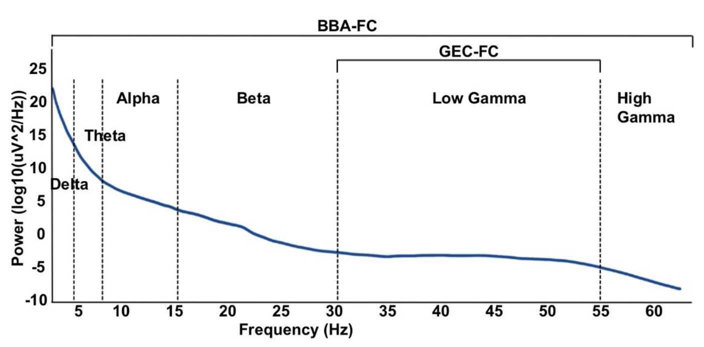
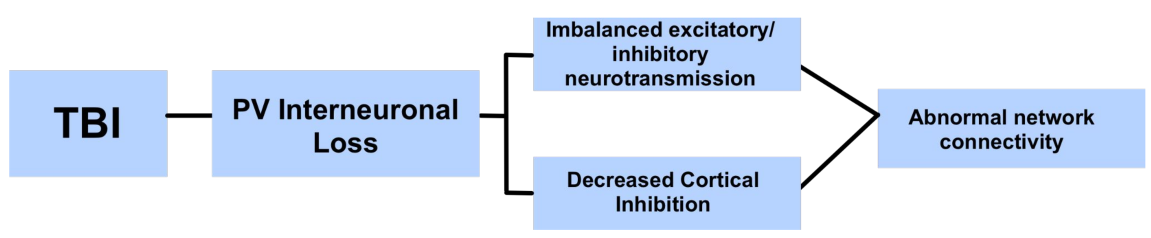
INTRODUCTION

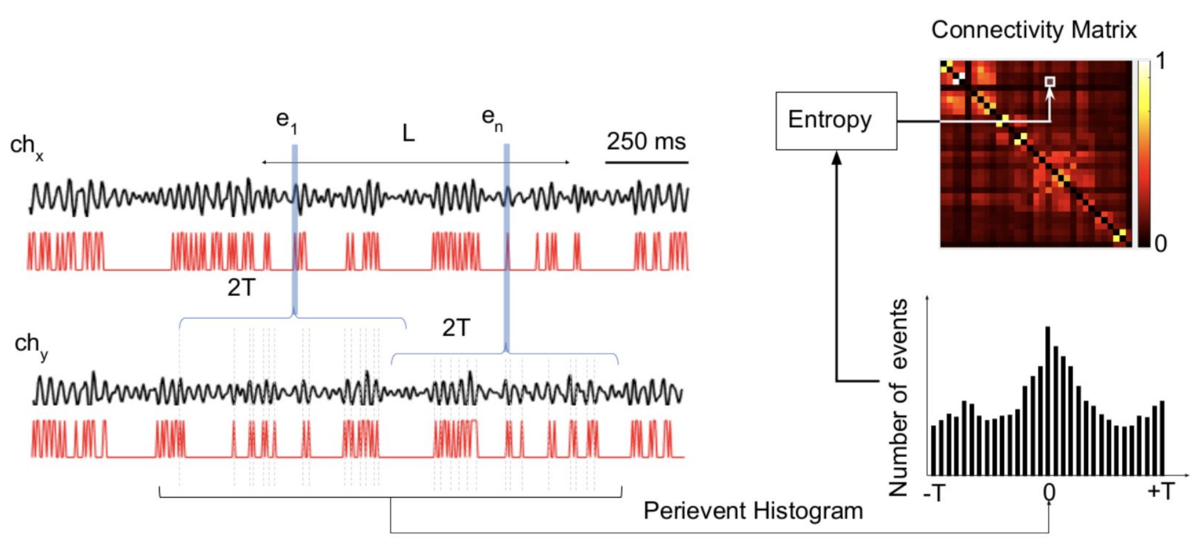
- Local Gamma Event Coupling functional connectivity (GEC-FC) reflects the synchronicity of regional gamma activity generated by fast spiking interneurons as a measure of functional connectivity.
- Global Broadband Amplitude Derived Functional Connectivity (BBA-FC) reflects local field potential voltage fluctuations at a broader range of frequencies and serves as a more global measure of neuronal activity.



- It is theorized that interneuronal cell loss results in global imbalanced excitatory/inhibitory neurotransmission, thus causing abnormalities in these networks
- GEC-FC and BBA-FC are hypothesized to reflect different neural substrates that underlie changes in functional connectivity after brain injury.
- **Aim 1:** To investigate the relationship between changes in local (GEC) and global (BBA) functional connectivity after fluid percussion injury using network-based analysis.
- **Aim 2:** To investigate the relationship between electrographic measures of seizure burden and measures of connectivity as precursors of potential epileptogenesis.

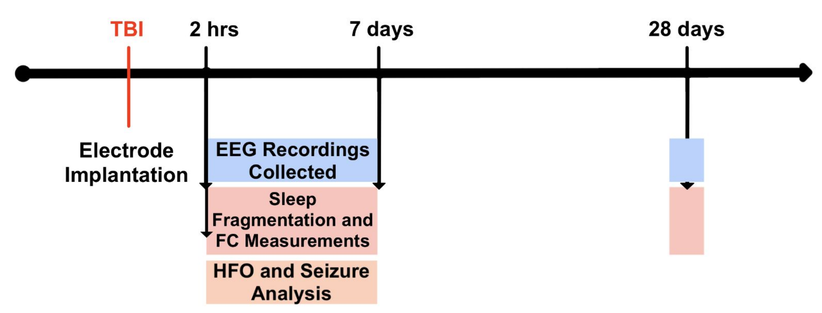


GAMMA EVENT COUPLING (GEC)



- Gamma oscillations arise from fast-spiking parvalbumin interneurons, which form highly connected local networks.
- GEC views gamma activity as a series of gamma events, calculating the synchronicity between electrodes using Shannon entropy to assess the strength of connectivity. GEC is stable within behavioral states and is sensitive to exhibitory and inhibitory changes in synaptic activity.
- This study used low gamma frequency (30-55Hz) for GEC measurements
- Strength of functional coupling for each pair of channels is represented in the connectivity matrix.

METHODS

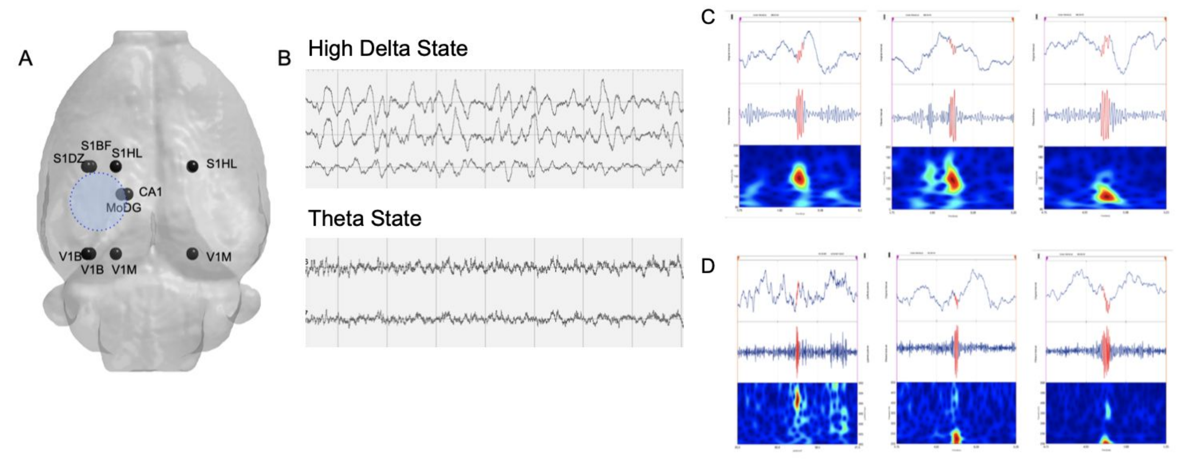


Experimental Procedure

- 12 Adult Male Sprague-Dawley Rats (6 TBI, 6 Sham)
- 5 mm diameter craniectomy with moderate-to-severe-level lateral FPI injury
- Electrodes (10) implanted in the occipital bone ipsilateral and contralateral (2) to injury, bipolar intracerebral microelectrodes in the anterior and posterior perilesional cortex (6), CA1 and dentate gyrus (2) hippocampal region
- Continuous EEG recordings for the first week after TBI and at 1 month post injury

Analysis

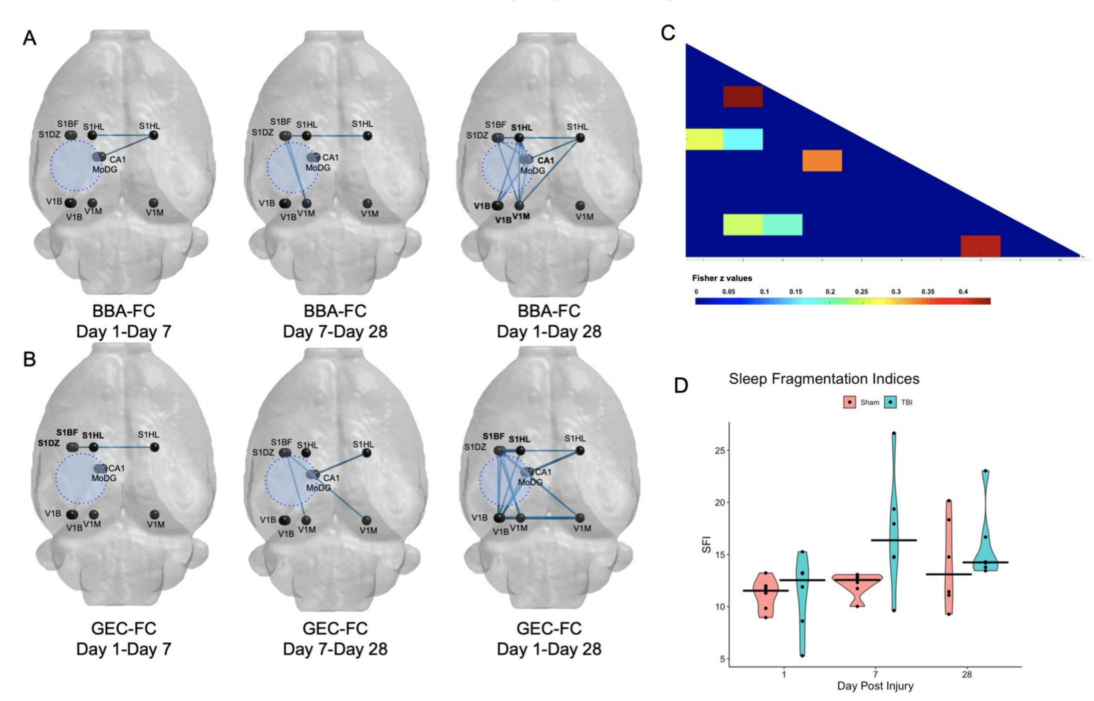
- Sleep-stage scoring of 24-hour EEG for high delta, theta, and awake epochs at 1, 7, and 28 days post injury (dpi) to calculate level of sleep-wake disturbance (Iber C, et al, 2007; AASM Manual for Scoring Sleep:1)
- Automatic high frequency oscillation (HFO) detection using RippleLab software and Short-Time Energy algorithm
- Broadband Connectivity FC, and Gamma Event Coupling FC calculated at 1, 7, 28 dpi, network connectivity measures were mapped onto a rat brain template
- Group differences in global connectivity measures calculated using graph theory (global efficiency, clustering coefficient, and characteristic path) were tested for using Network Based Statistics, p<0.01



Representative Electrode Placement, EEG Traces, and HFO Analysis

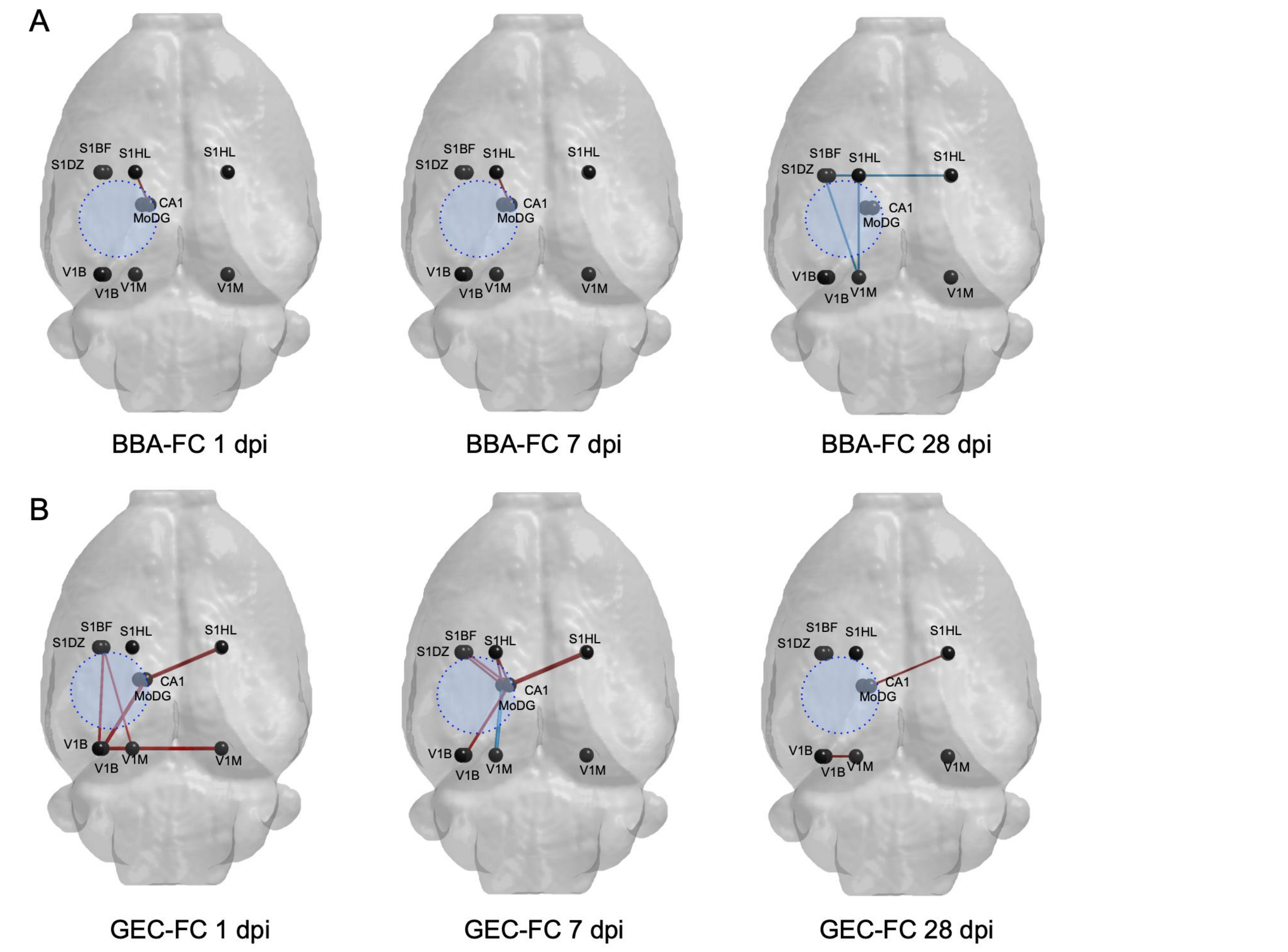
[A] Map of electrode placements (black spheres) and the approximate injury location (blue shaded circle)
[B] Representative EEG traces of delta and theta states used in the 24-hour sleep stage scoring and functional connectivity analysis
[C-D] Examples of ripple **[C]** and Fast Ripple **[D]** activity, distinguishable by their unique patterns of oscillation, as accentuated by the accompanying heat maps

RESULTS



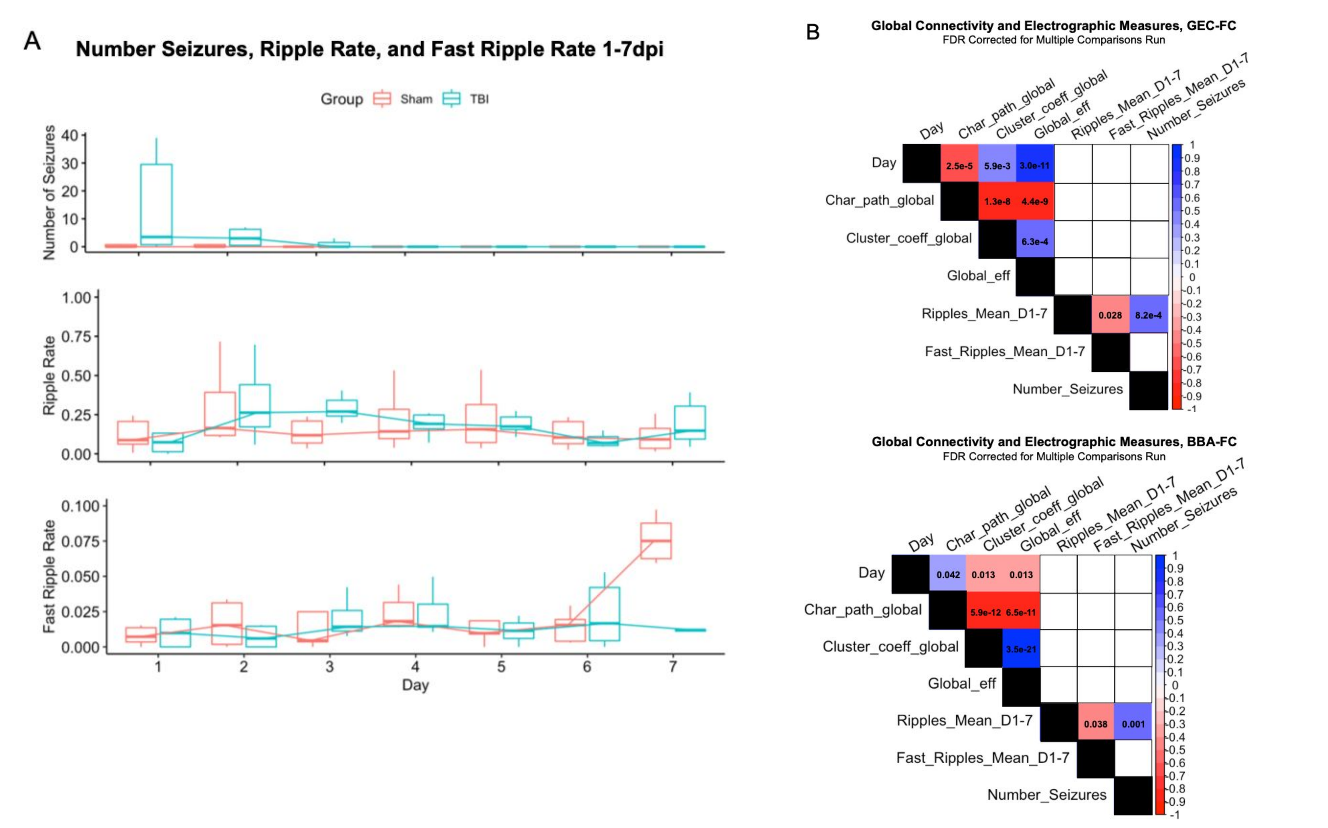
Temporal Changes in Functional Connectivity within the TBI Group

Statistically different edge connectivity between electrodes comparing TBI changes over time
KEY: Red: FC over time is TBI Earlier<Later day, Blue: FC over time is Earlier>Later day (p<0.01, NBS)
[A] BBA-FC and **[B]** GEC-FC within the TBI group decreased over time, particularly across hemispheres (p<0.01).
[C] Representative matrix of GEC-FC differences, colored boxes indicate significant edge connections between indicated electrodes.
[D] Sleep Fragmentation (SF) was significantly increased (p<0.05) in TBI rats at 7dpi and trended towards an increase over time. There was no significant correlation between degree of SF and FC changes.



Group Level Functional Connectivity Changes Post-Injury

Statistically different edge connectivity between electrodes comparing TBI and sham groups at 1, 7, and 28dpi for: **[A]** BBA-FC and **[B]** GEC-FC networks.
KEY: Red: FC is TBI>Sham, Blue: FC is TBI<Sham (p<0.01, NBS); Black sphere: electrode position
[A] BBA-FC analysis showed ipsilateral sensory-cortex-to-CA1 hippocampus hyperconnectivity that transformed to ipsilateral deficits at 28 dpi.
[B] GEC-FC analysis demonstrated larger regions of early and persistent ipsilateral connectivity compared to shams. GEC-FC weakened over time but remained hyperconnected compared to sham.



Relationship between Electrographic measures (Seizures, Ripples, & Fast Ripples) and Global Connectivity Measures

[A] Seizures peaked 1 dpi but decreased to sham level. Ripple Rate remained relatively similar between groups across timepoints. Fast Ripple Rate demonstrated variation between groups, with a general trend for similarity between groups.
[B] Correlation plot between Day, Global Connectivity metrics, and HFO Ripples, Fast Ripples, and Number of Seizures (p<0.05, FDR corrected for multiple correlations run). There was no significant correlation in either GEC-FC or BBA-FC analysis between global connectivity and ripples, fast ripples, or number of seizures.

SUMMARY & CONCLUSIONS

- Both BBA-FC and GEC-FC demonstrated initial hyperconnectivity after TBI.
- BBA-FC revealed ipsilateral FC deficits at 28 dpi but GEC-FC showed prolonged hyperconnectivity that weakened over time.
- Differences in BBA and GEC-derived measures of connectivity may reflect different underlying mechanisms, and one possibility is that interneuronal changes drive GEC after injury.

References:
Shamas et al, 2022; 2023; eNeuro:9
Iber C, et al, 2007; AASM Manual for Scoring Sleep:1
Buzsáki and Wang; Annual review of neuroscience:35:203-225
Funding: U54-NS100064 (EpiBios4Rx) & UC Brain Injury Research Center

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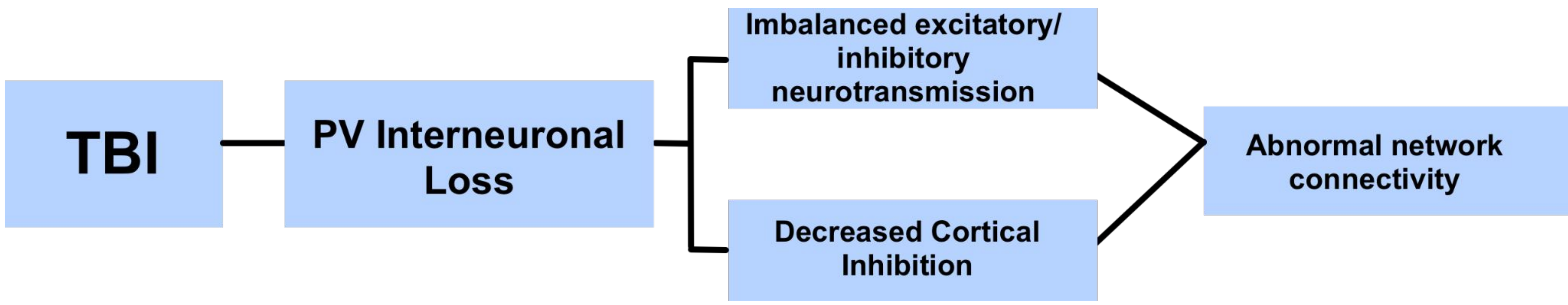
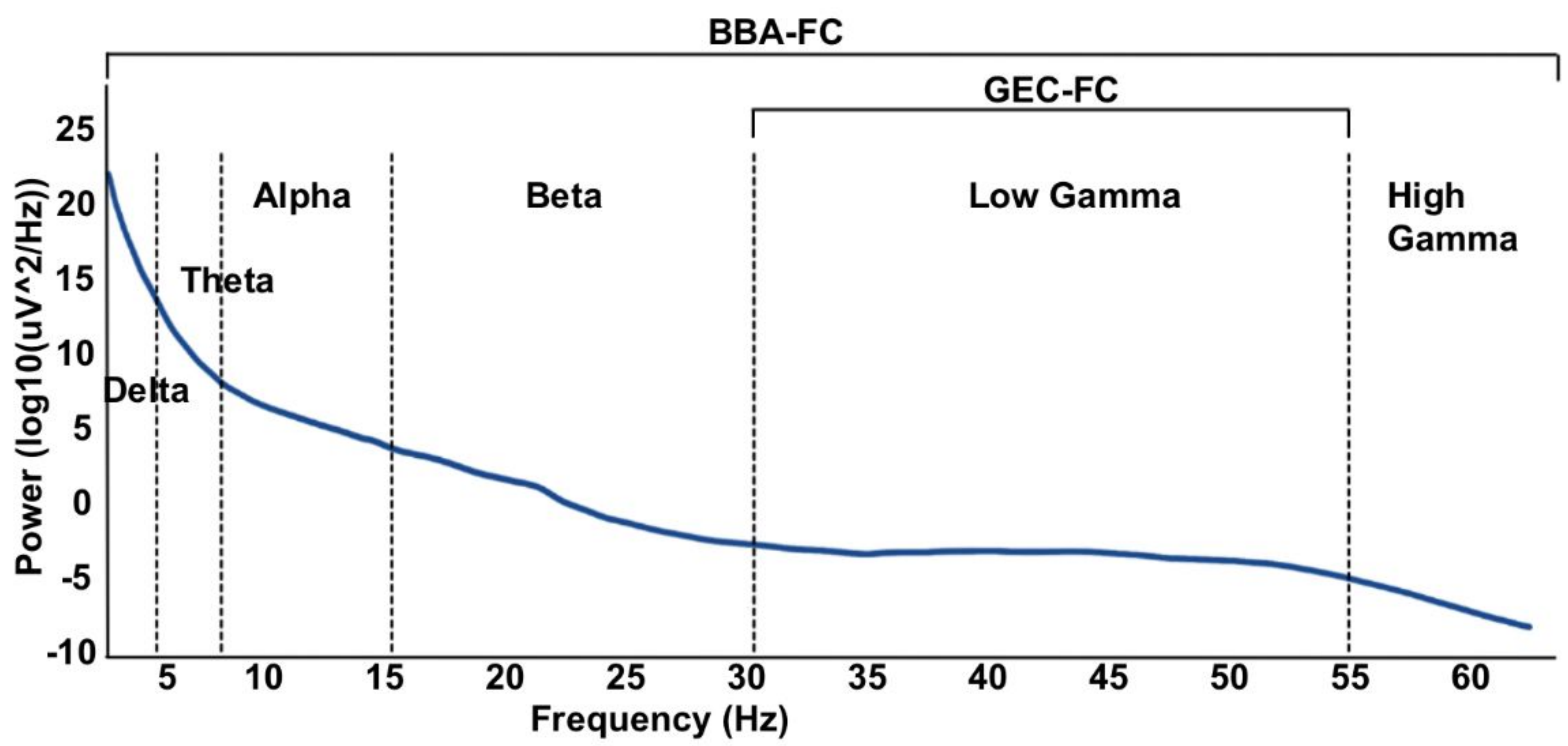
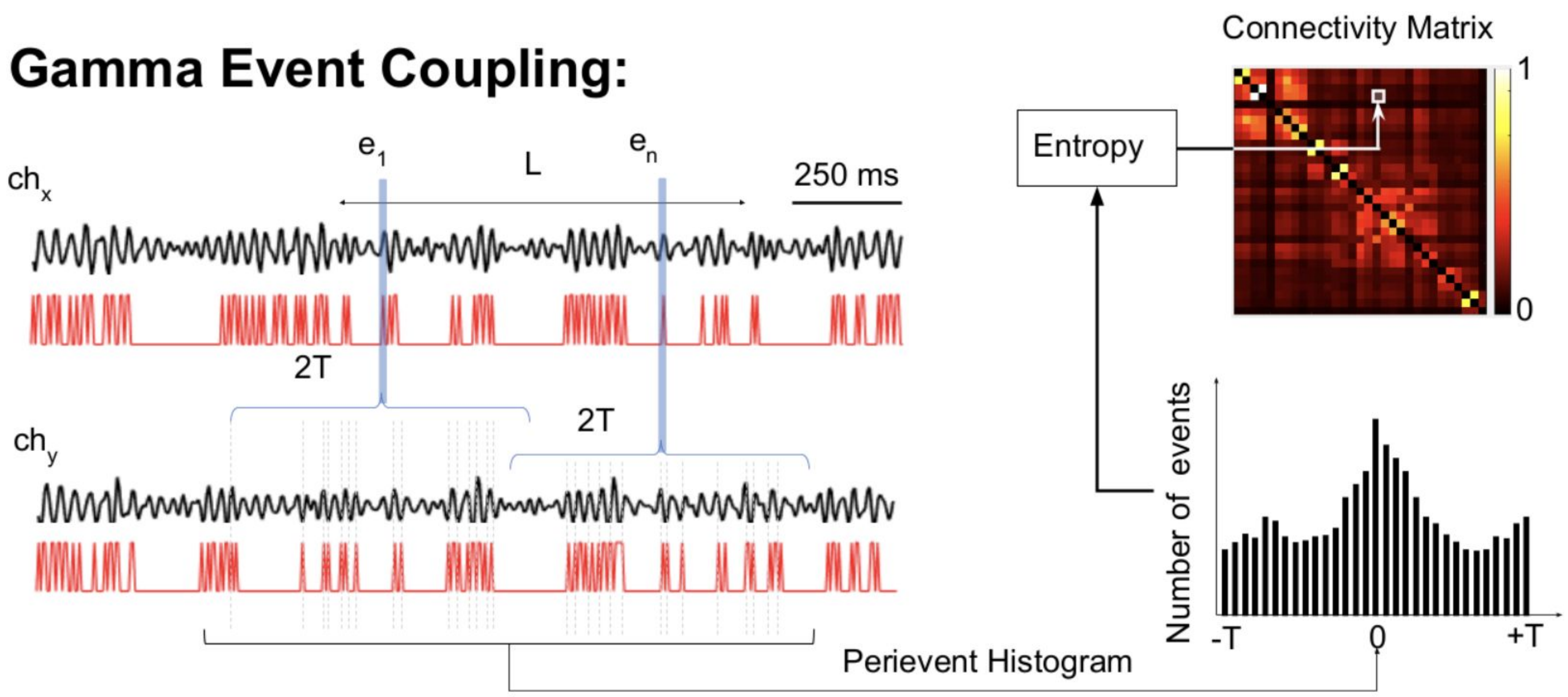
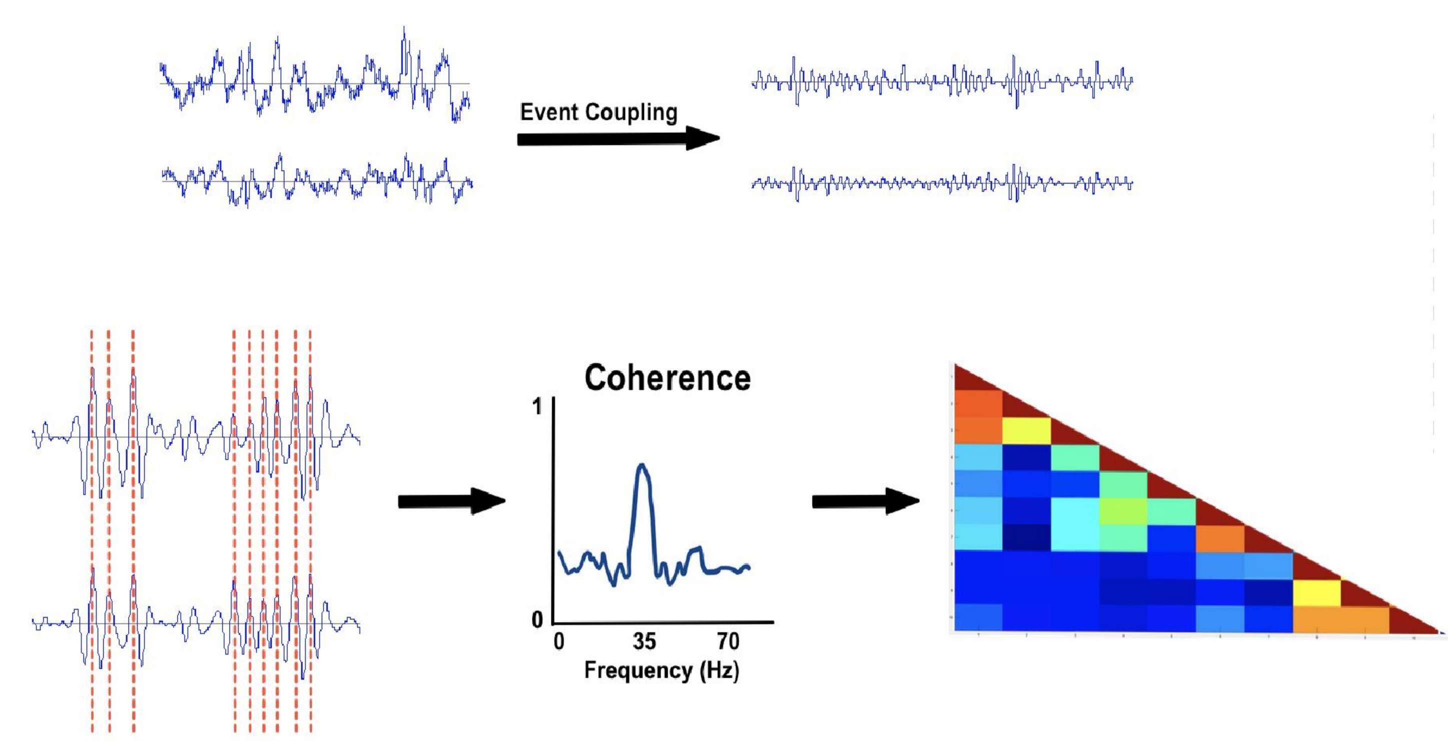




Gamma Event Coupling





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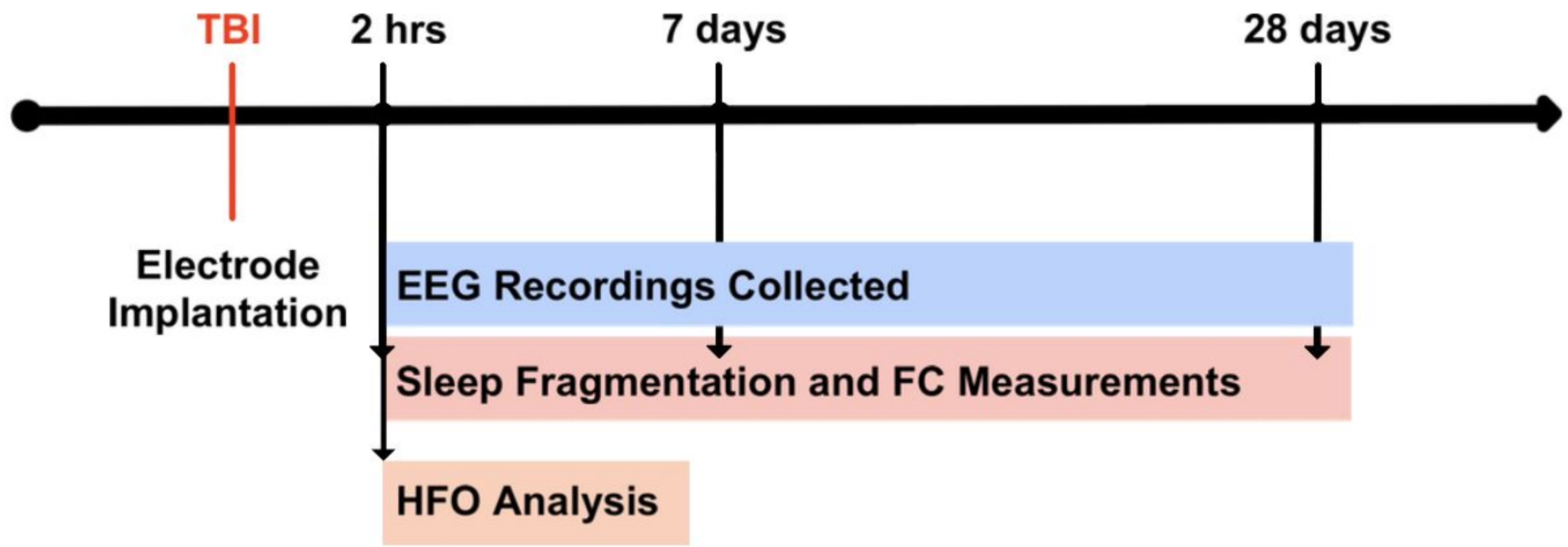

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- 5 mm diameter craniectomy with moderate-severe lateral FPI injury (apnea scores 24-45 seconds)
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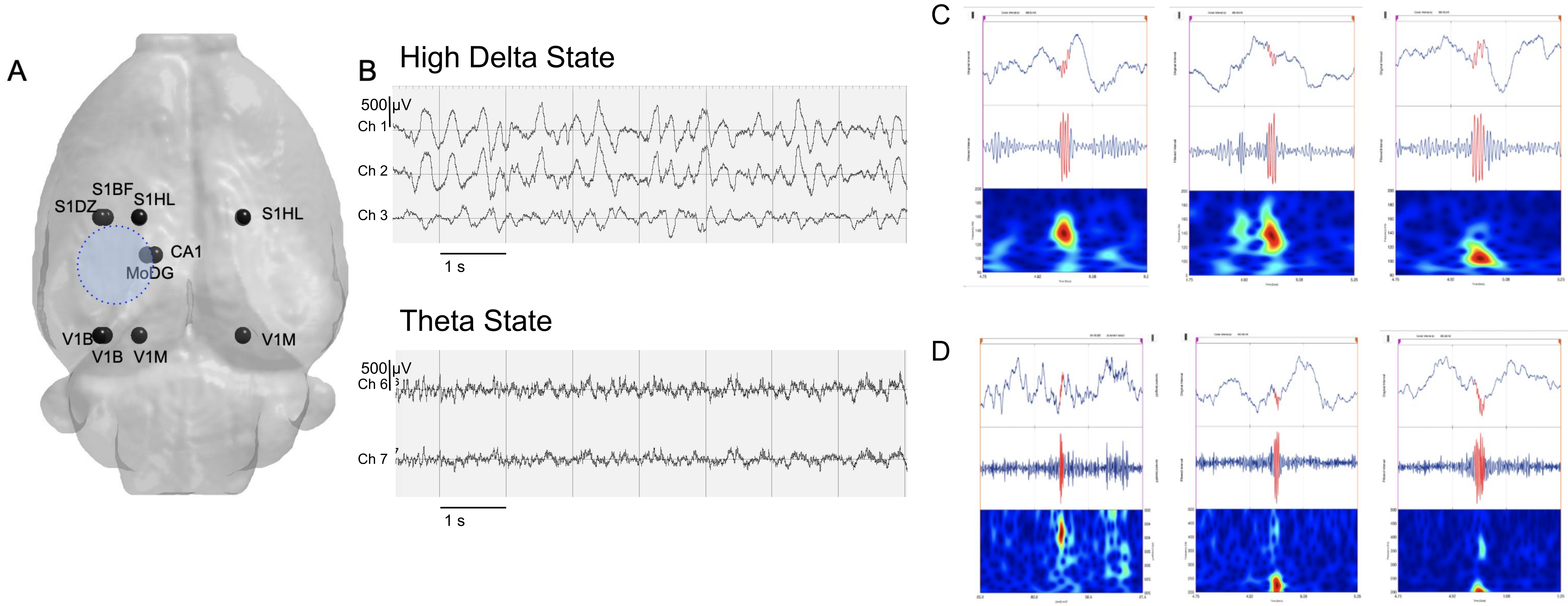
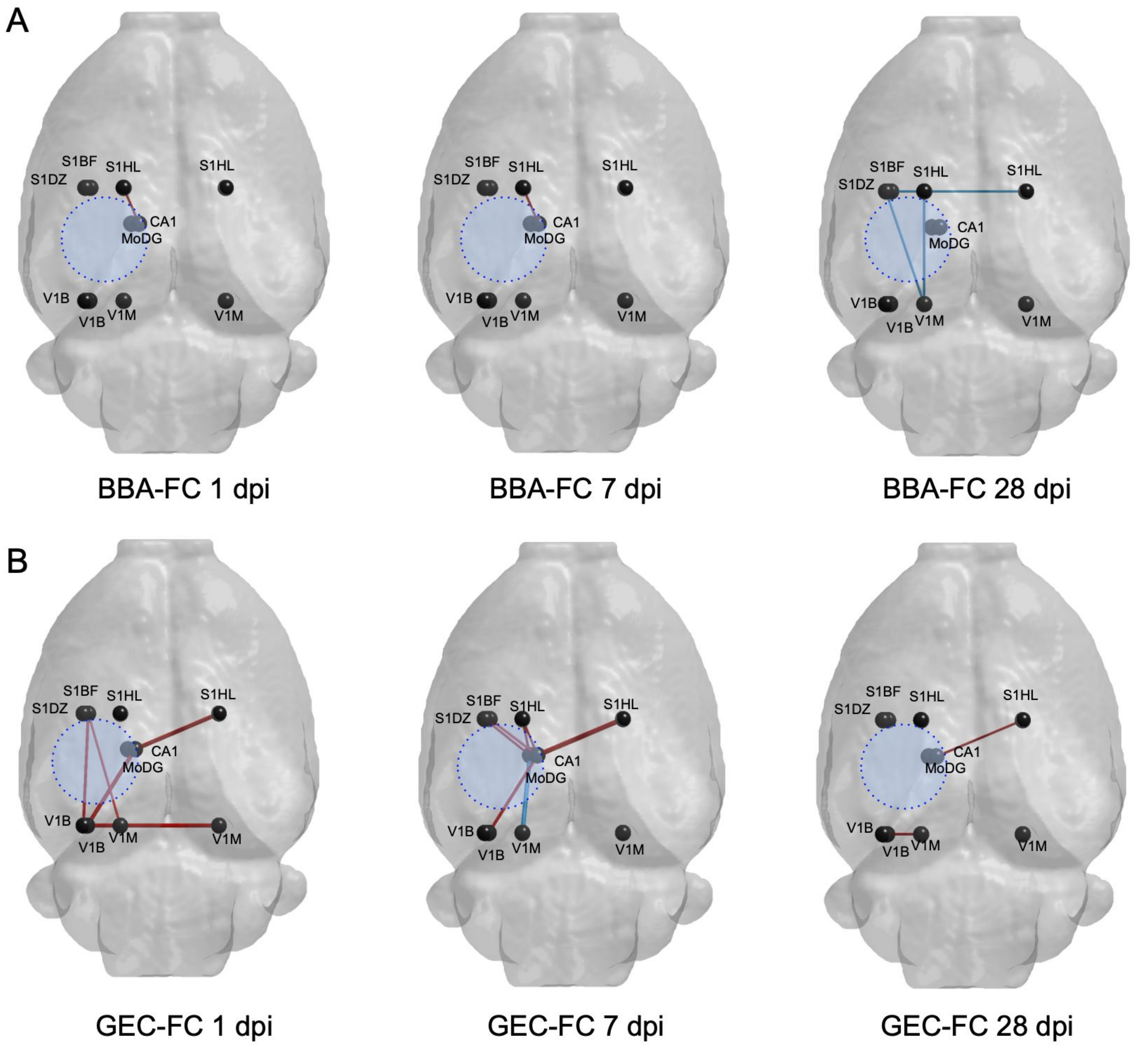
Data Analysis

- Sleep-stage scoring of 24-hour EEG for high delta, theta, and awake epochs at 1, 7, and 28 dpi to calculate level of sleep-wake disturbance
- Automatic high frequency oscillation (HFO) detection using RippleLab software and Short-Time Energy algorithm
- Broadband Connectivity FC, and Gamma Event Coupling FC calculated at 1, 7, 28 dpi, network connectivity measures mapped onto rat brain template
- Global Connectivity analysis of global efficiency, clustering coefficient, and characteristic path using Network Based Statistics, $p<0.01$



- A) Map of electrode placement with shaded circle representing injury location
- B) Representative EEG trace of delta and theta states used in 24 hour sleep stage scoring and functional connectivity analysis
- C-D) Example of ripple (C) and Fast Ripple (D) activity in Ripple Lab software with distinguishable oscillations and localized heat map

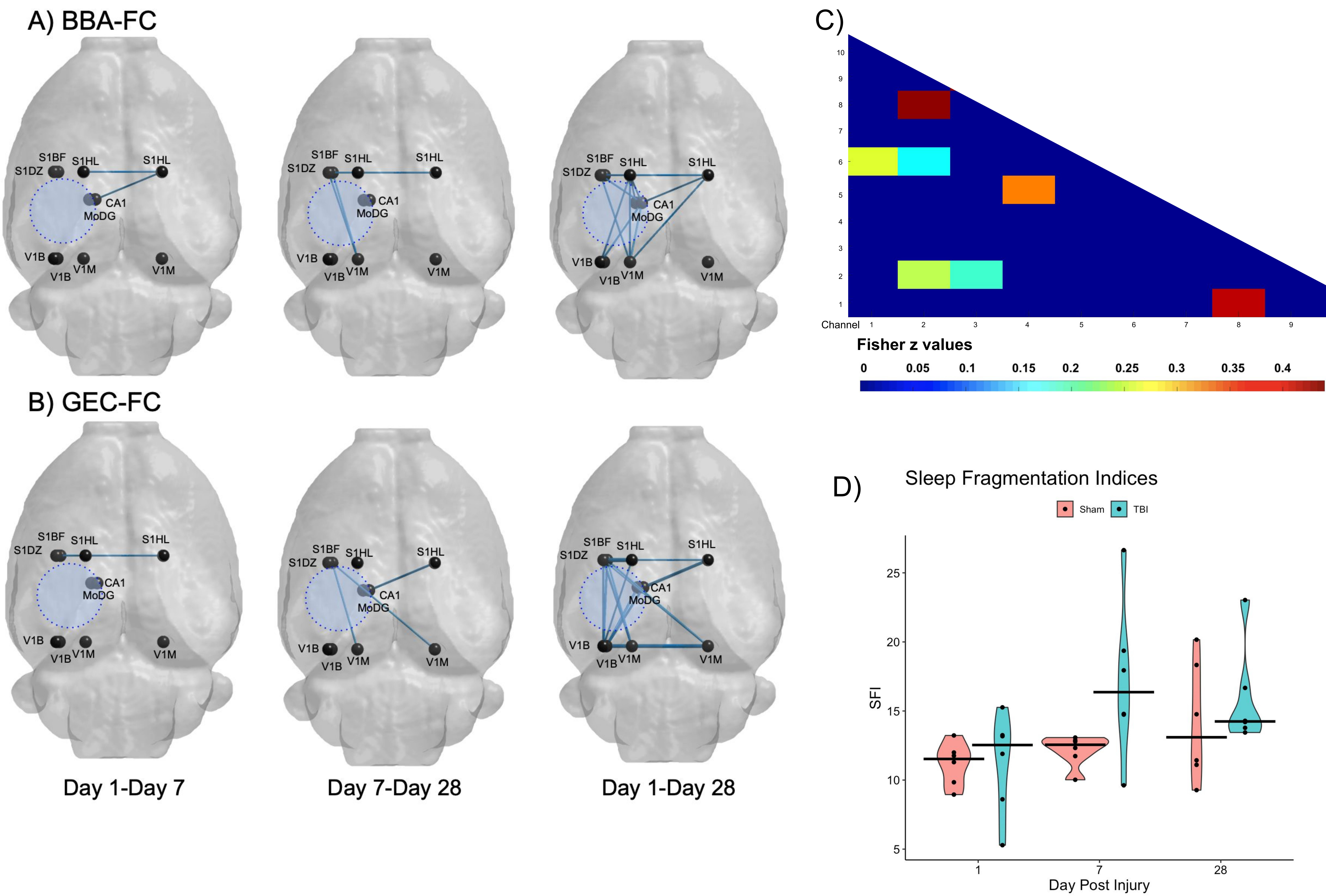


Statistically different edge connectivity between electrodes comparing TBI and sham groups at 1, 7, and 28dpi for: [**A**] BBA-FC and [**B**] GEC-FC networks.

<u>KEY</u>: Red: FC is TBI**>**Sham, Blue: FC is TBI**<**Sham (p<0.01, NBS); Black sphere: electrode position

[**A**] BBA-FC analysis showed ipsilateral sensory-cortex-to-CA1 hippocampus hyperconnectivity that transformed to ipsilateral deficits at 28dpi.

[**B**] GEC-FC analysis demonstrated larger regions of early and persistent ipsilateral connectivity compared to shams. GEC-FC weakened over time but remained hyperconnected compared to sham.



Statistically different edge connectivity between electrodes comparing changes over time, TBI vs Sham.

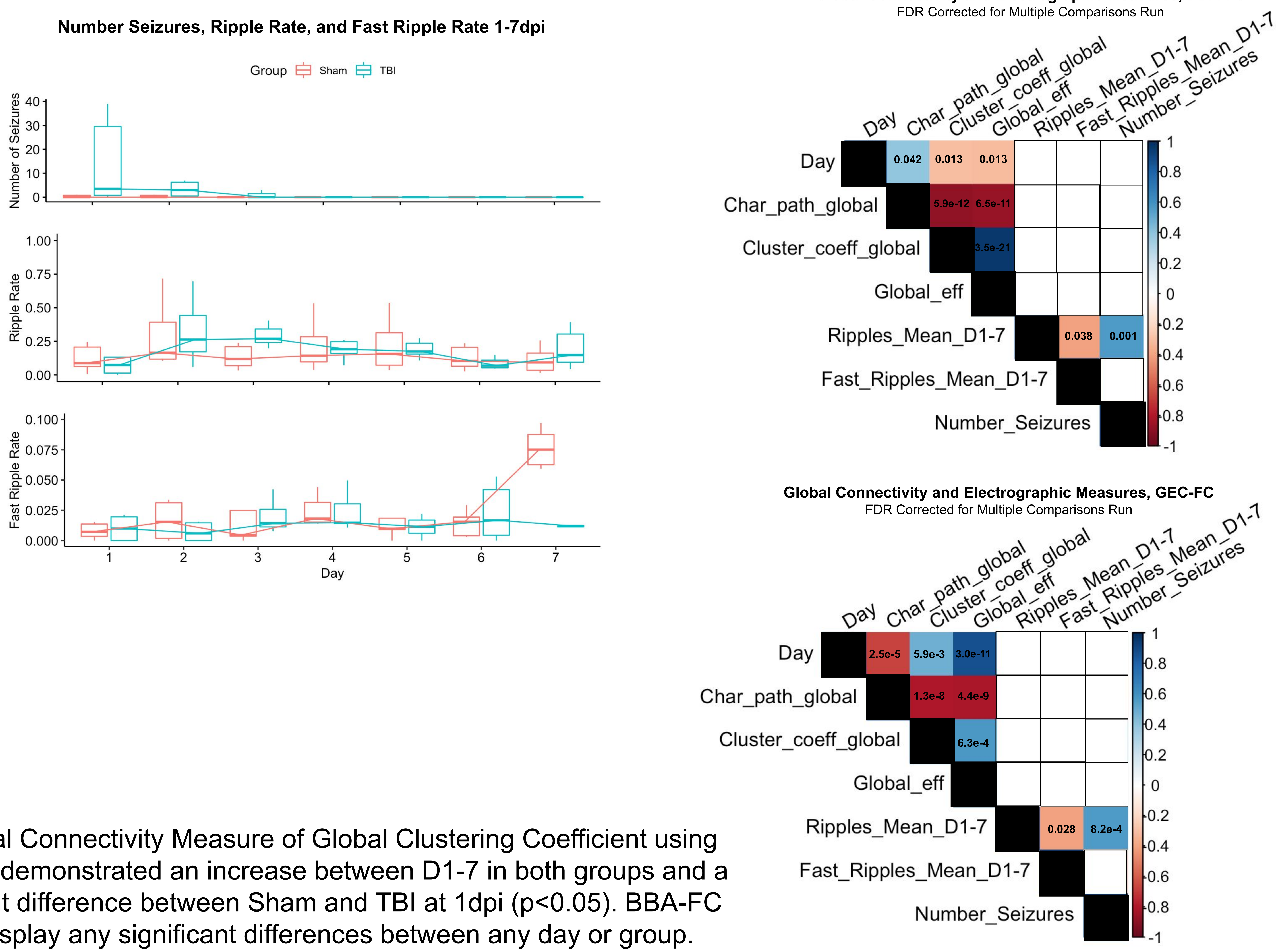
<u>KEY</u>: Red: FC over time is TBI**>**Sham, Blue: FC over time is TBI**<**Sham ($p<0.01$, NBS)

[**A**] BBA-FC and [**B**] GEC-FC within the TBI group decreased over time, particularly across hemispheres ($p<0.01$).

[**C**] Representative matrix of GEC-FC differences, colored boxes indicate significant edge connections between indicated electrodes.

[**D**] Sleep Fragmentation was significantly increased ($p<0.05$) in TBI rats at 7dpi and trended towards an increase over time.

A B



[A] Global Connectivity Measure of Global Clustering Coefficient using GEC-FC demonstrated an increase between D1-7 in both groups and a significant difference between Sham and TBI at 1dpi (p<0.05). BBA-FC did not display any significant differences between any day or group.
[B] Correlation plot between Day, Global Connectivity metrics, and HFO Ripples, Fast Ripples, and Number of Seizures. There was no significant correlation in either GEC-FC or BBA-FC analysis between global connectivity and ripples, fast ripples, or number of seizures.

SUMMARY

- Interneuronal changes in the brain can be measured by gamma event coupling functional connectivity. BBA-FC and GEC-FC both demonstrated initial hyperconnectivity after TBI with BBA-FC revealing ipsilateral FC deficits at 28 dpi and GEC-FC showing maintained hyperconnectivity that weakened over time.

- The difference in FC between BBA and GEC-derived measures reflects different mechanisms driving FC changes after injury due to interneuronal changes. Future electrographic analysis of seizure data will be used to examine the extent to which FC changes are related to prior electrical activity of the brain.