Statistical Approaches for Neural Oscillation and Social Behavior Analysis in the Ventral Tegmental Area

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INTRODUCTION

The ventral tegmental area (VTA) is a key hub in the brain's reward circuitry, projecting extensively to the prefrontal cortex (PFC). Communication between the VTA and PFC is critical for social behavior and is disrupted in conditions such as addiction and depression[1].

Although ethanol (EtOH) is known to alter neuronal excitability and neurotransmitter release, its impact on the dynamic coordination between VTA and PFC remains poorly characterized. In particular, how acute EtOH exposure modulates directional information flow, cross-frequency coupling, and social behavior-related oscillatory dynamics is still unclear.

This study investigates how acute EtOH affects neural oscillations and connectivity between the VTA and PFC. We focus on directionality using Granger causality (GC), and synchronization using intra- and inter-region phase-amplitude coupling (PAC).These analyses aim to identify circuit-level signatures of ethanol's effects on behavior and neural coordination, with potential implications for understanding alcohol-related neuropsychiatric conditions.

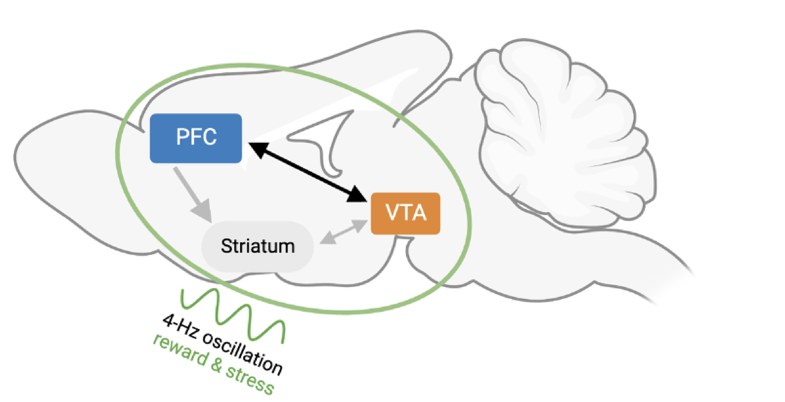

Figure 1. PFC–VTA Circuit Involved in Reward and Stress-Related Oscillations

METHODS

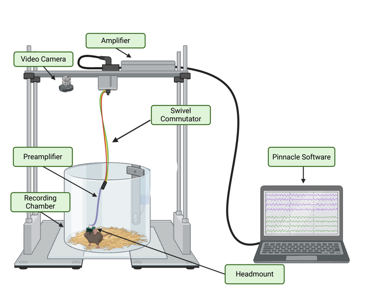

Figure 2. Experimental Protocal of Open Field Paradigm

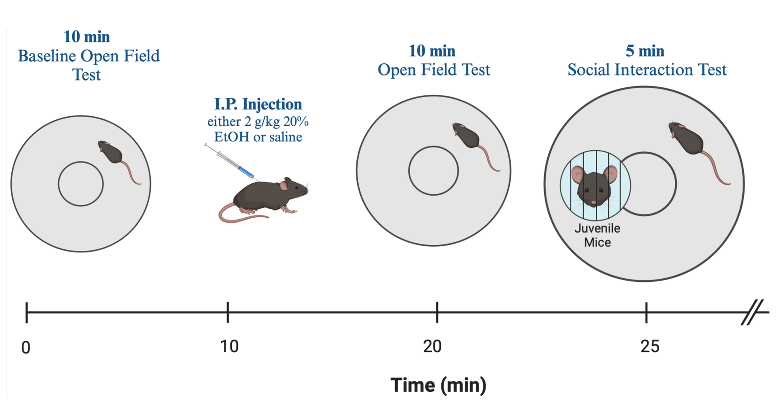

Figure 3. Experimental Timeline of Ethanol and Social Interaction Testing

LFP signals were recorded via intracranial electrodes implanted in the VTA and PFC of freely moving mice during open-field exploration and social interaction tasks. Signals were collected under baseline and ethanol conditions.

1. **Preprocessing**: LFPs were notch-filtered (60 Hz), bandpass-filtered (theta: 4–12 Hz, gamma: 30–40 Hz), z-scored, and segmented by condition and time.
2. **Granger Causality**: Directional connectivity (PFC↔VTA) was computed in the theta band and summarized with confidence intervals.
3. **PAC Analysis**: iPAC (within-region) and irPAC (cross-region; e.g., VTA phase → PFC amplitude) were quantified across theta-gamma ranges.
4. **Behavioral Segmentation**: Social interaction periods were identified by tracking proximity between the experimental mouse and a stimulus conspecific in the arena. LFP data during these epochs were extracted for condition-specific analysis.
5. **Statistics**: Group means ± SEM/CI; repeated-measures comparisons used mixed-effects models in MATLAB (brainstorm) and R.

RESULTS

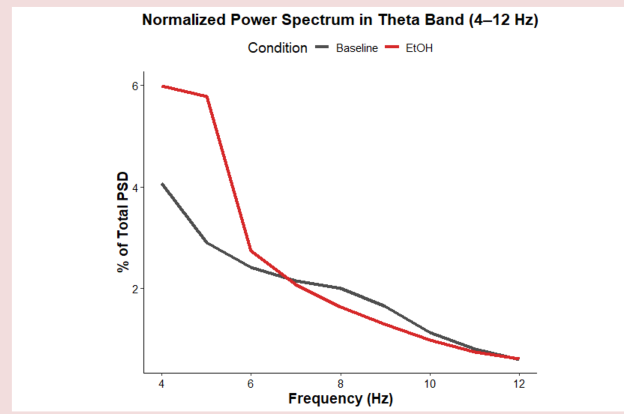

Figure 4. Normalized Power Spectrum in the Theta Band
Elevation in PSD in lower frequency potentially reflects increased neural synchronization during EtOH exposure.

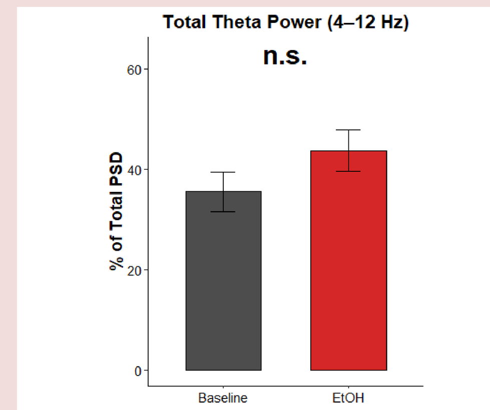

Figure 5. Total Normalized Theta Power Across Conditions
Normalized theta band power was significantly higher in EtOH condition.

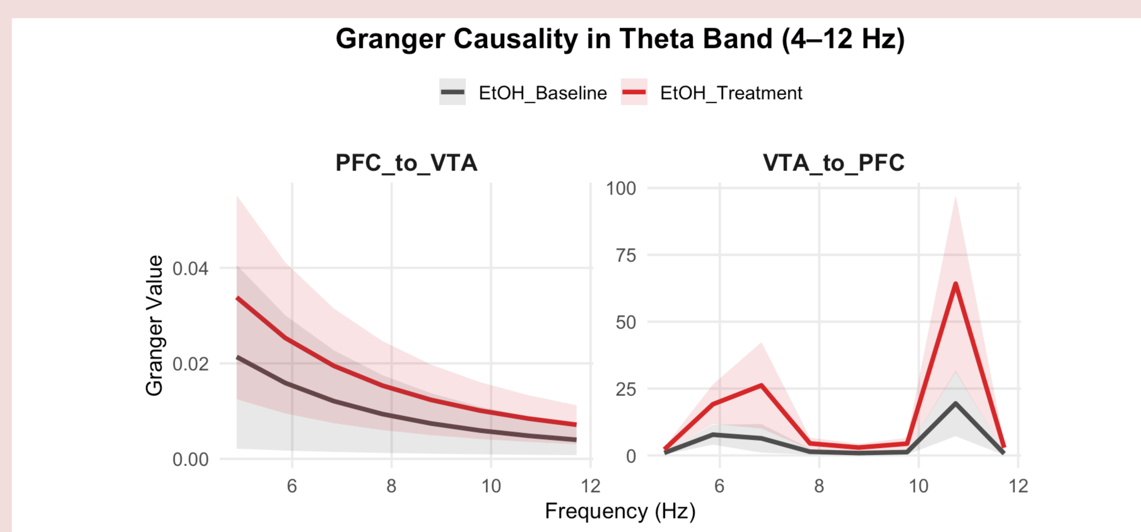

Figure 6. Granger Causality in Theta Band (4–12 Hz)
Directional connectivity between PFC and VTA under ethanol (EtOH) and baseline conditions. Mean frequency-resolved Granger causality values are shown with shaded 95% confidence intervals.

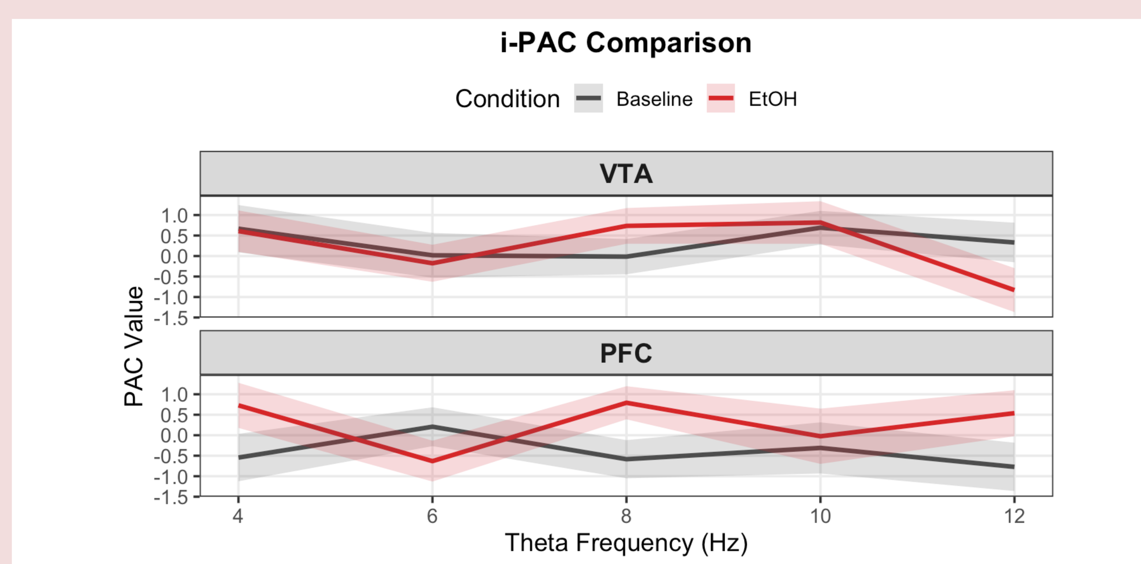

Figure 7. Intra-Regional Phase-Amplitude Coupling (iPAC) under Ethanol
EtOH enhanced PAC in both regions, with a greater effect observed in the VTA. Lines represent mean PAC values with shaded SEM.

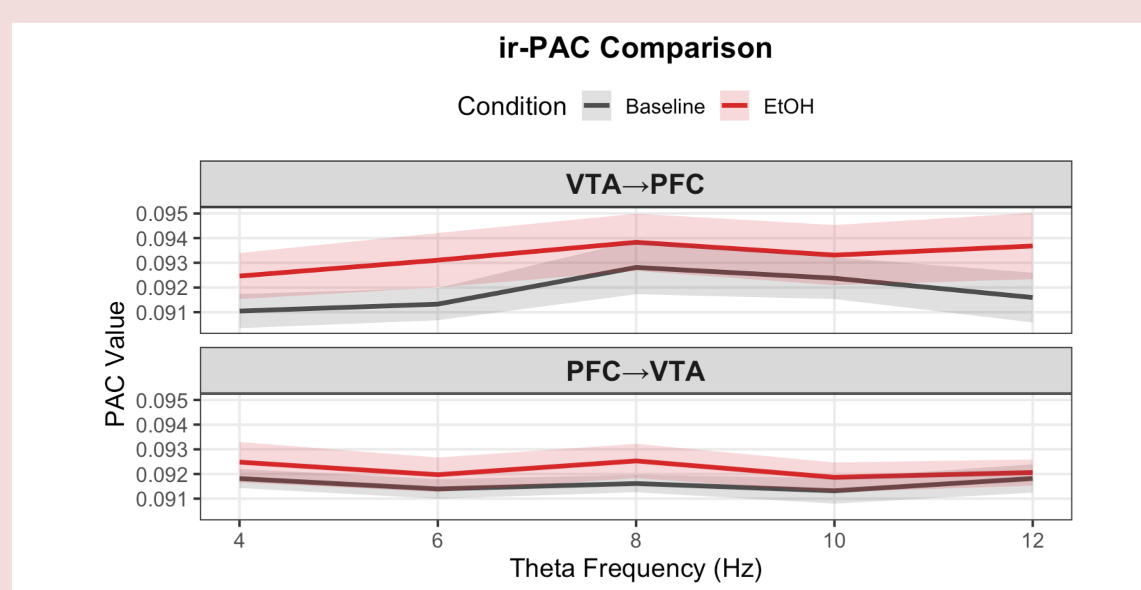

Figure 8. Inter-Regional Phase-Amplitude Coupling (irPAC) under Ethanol
EtOH modestly elevated irPAC in both directions. Lines represent mean PAC values with shaded SEM.

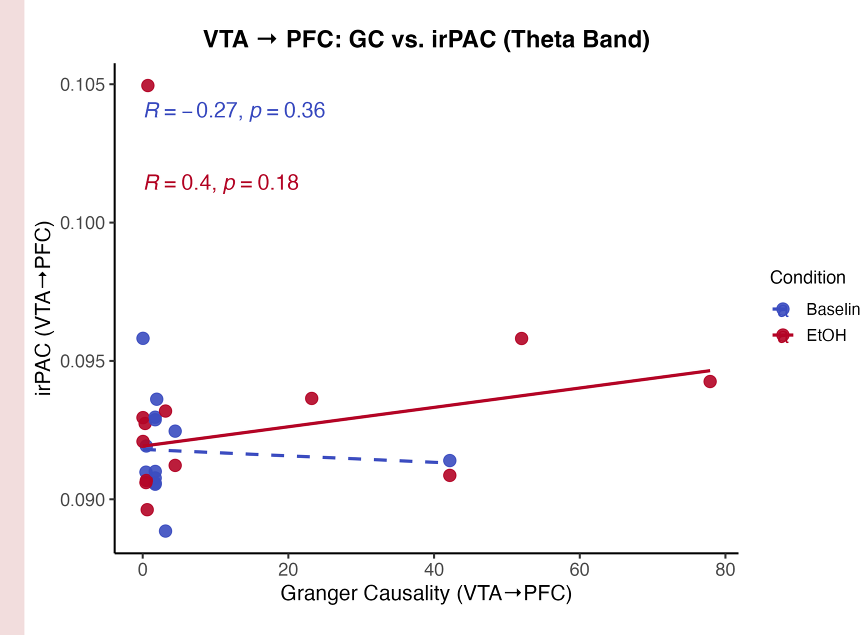

Figure 9. Relationship between Granger Causality and inter-regional PAC in the VTA → PFC pathway
EtOH enhances irPAC level over the increase of Granger Causality. Robust linear regression fitted for each condition, and Spearman correlation coefficients (ρ) with p-values are shown.

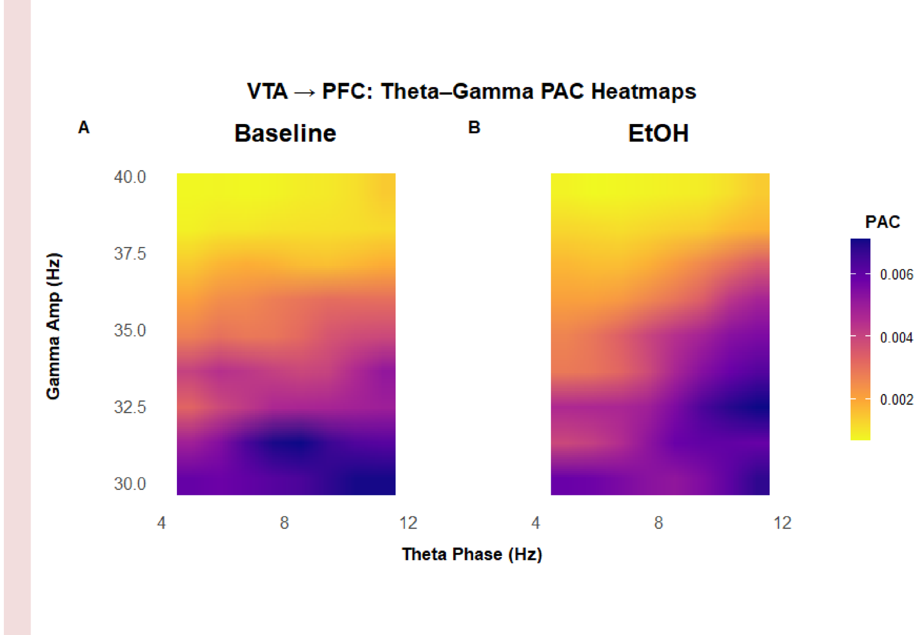

Figure 10. VTA→PFC theta–gamma PAC Heatmap under baseline and EtOH
PAC Heatmap show VTA theta phase (4–12 Hz) modulating PFC gamma amplitude (30–40 Hz). Ethanol increased PAC strength compared to baseline, especially in lower gamma band (around 33 Hz) suggesting enhanced cross-regional coupling.

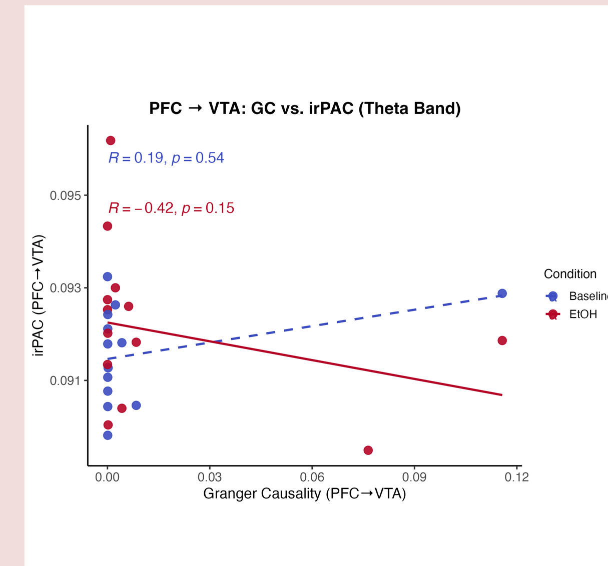

Figure 11. Relationship between Granger Causality and inter-regional PAC in the PFC →VTA pathway
Each point represents an individual subject. Separate robust regression lines were fitted for Baseline and EtOH conditions, with Spearman correlation results annotated.

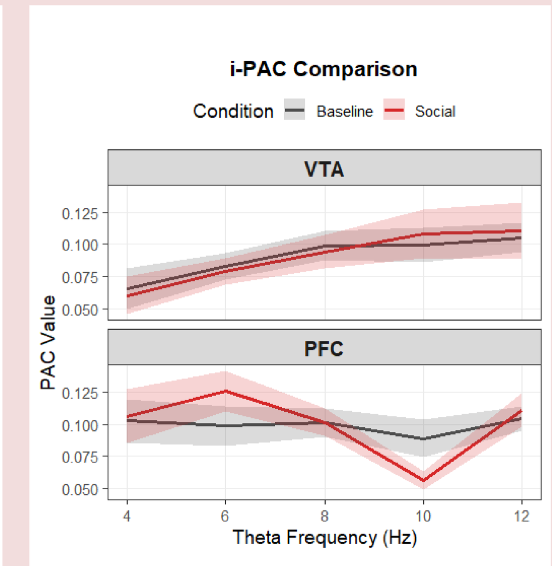

Figure 12. Intra-Regional PAC under Social Condition
Social interaction enhanced theta–gamma coupling in both regions, with PFC showing stronger frequency-dependent modulation. Shaded ribbons denote SEM.

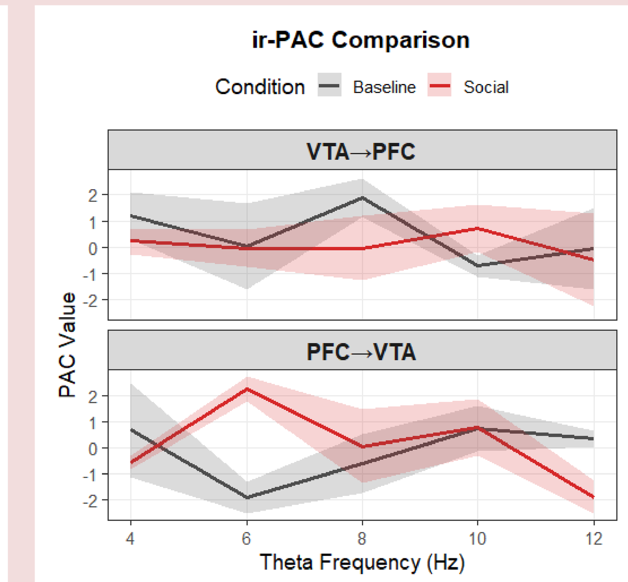

Figure 13. Inter-Regional PAC under Social Condition
Social interaction reduced ir-PAC in the VTA→PFC pathway, especially around 8 Hz, while increasing it in the PFC→VTA direction around 6 Hz. Shaded ribbons denote SEM.

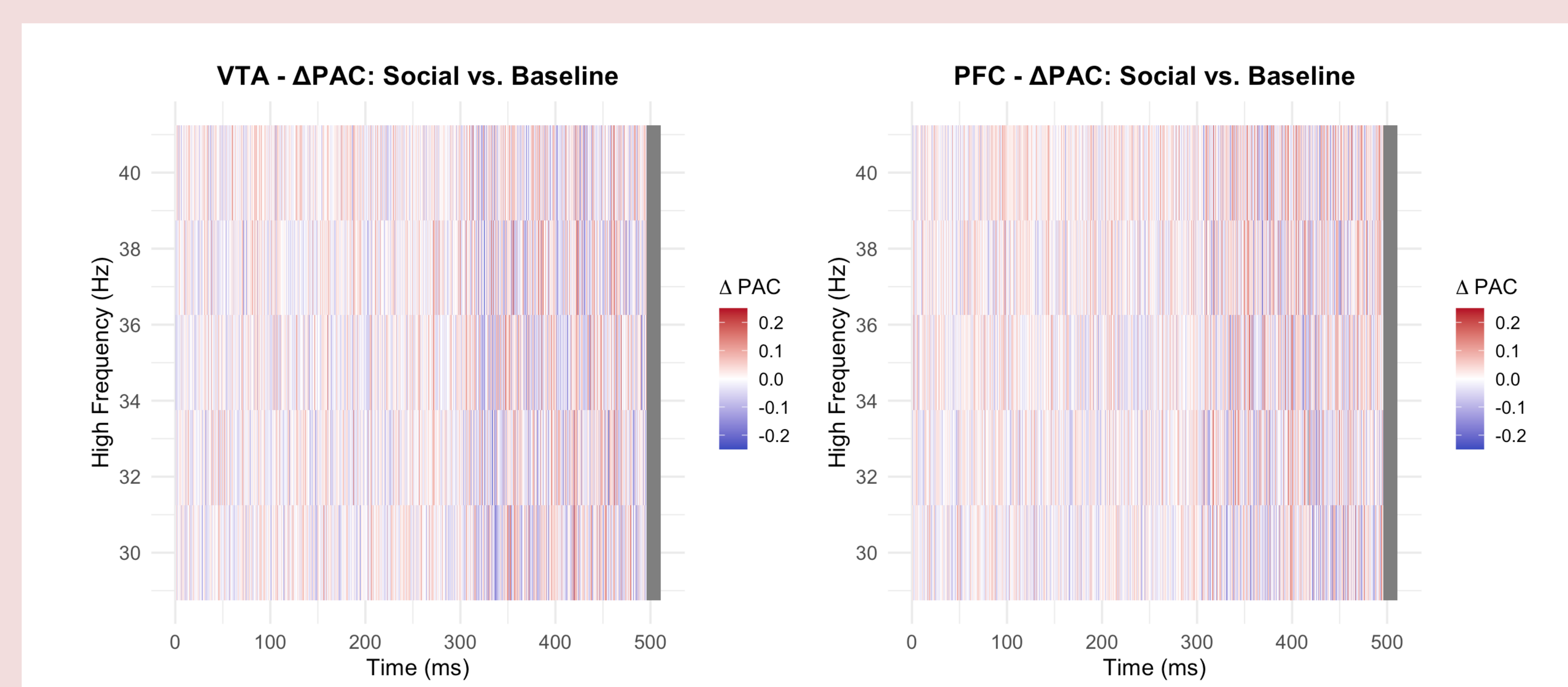

Figure 14. ΔPAC Maps (Social – Baseline)
Change in PAC (ΔPAC) from baseline to social condition in VTA and PFC. Red regions indicate PAC increases during social interaction; blue regions represent reductions.

CONCLUSION

Firstly, Granger causality analysis indicated that ethanol significantly reduced excitatory projections from the VTA to the PFC in the theta band (4 - 12 Hz), indicating suppressed bottom-up signaling. In contrast, PFC→VTA connectivity remained relatively stable. The result was directed by increases in intra-regional PAC (iPAC) in both VTA and PFC, suggesting that ethanol enhances local frequency integration. Inter-regional PAC (irPAC) further indicated a reduction in VTA→PFC coupling, accompanied by a slight increase in the PFC→VTA direction, supporting the hypothesis of directional reorganization under ethanol.

Secondly, correlational analyses between GC and irPAC suggested a potential functional trade-off: GC and irPAC were negatively associated in the VTA→PFC direction at baseline and positively correlated under ethanol, with the reverse relationship observed in the PFC→VTA direction; however, none of these reached statistical significance.

Thirdly, during ethanol-injected social interaction, iPAC further elevates in both regions. PFC exhibited frequency-dependent modulation (notably a dip near 10 Hz), along with increased PAC variability. irPAC analyses showed enhanced PFC→VTA coupling around 6 Hz, while VTA→PFC remained unstable.

Together, these findings highlight that both ethanol and social stimuli modulate VTA–PFC coordination in complex, pathway-specific ways. This work displays the dynamic interplay between connectivity and oscillatory integration, with implications for understanding social behavior, addiction, and neuropsychiatric dysfunctions.

REFERENCES

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[2] Nandi, B., Swiatek, P., Kocsis, B., & Ding, M. (2019). Inferring the direction of rhythmic neural transmission via inter-regional phase-amplitude coupling (ir-PAC). *Scientific reports*, *9*(1), 6933.

ACKNOWLEDGEMENTS

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