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Gamma Band Functional Connectivity in Olfactory-Prefrontal Pathways During Anxious States

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ABSTRACT

Background: Anxiety disorders affect nearly 30% of adults worldwide and involve dysfunctional threat processing, yet the neural mechanisms remain partially understood. This study investigates the role of gamma-band oscillations in olfactory bulb (OB) and prefrontal cortex (PFC) interactions during anxiety-like behavior.

Methods: Chronic local field potentials were recorded simultaneously from OB and PFC in male Wistar rats (n = 12) during Elevated Plus Maze (EPM) and Open Field (OF) tasks. Directional functional connectivity was assessed using Granger causality analysis across low (30–50 Hz) and high (50–100 Hz) gamma frequency bands.

Results: Results showed significant feedforward (OB→PFC) connectivity in both gamma bands during behavioral tasks. The directional influence from OB to PFC was significantly greater than the reverse pathway across conditions. These findings indicate robust bottom-up communication between OB and PFC.

Conclusion: Gamma oscillations appear to mediate sensory-to-prefrontal information transfer during threat evaluation, with low gamma supporting interregional coordination and high gamma facilitating localized processing within the PFC. This frequency-dependent coupling highlights potential neural mechanisms underlying anxiety.

Keywords: Anxiety, Gamma oscillations, Granger causality.

Introduction

Anxiety disorders constitute a class of debilitating mental health conditions characterized by excessive and persistent threat response mechanisms that persist in non-threatening environments, leading to significant neurocognitive dysfunction. diagnostic frameworks classify these disorders among the most widespread neuropsychiatric ailments, imposing substantial individual suffering and socioeconomic burdens through impaired occupational functioning and increased medical resource utilization. Epidemiological research demonstrates that approximately 30% of the global adult population will experience clinically significant anxiety symptoms during their lifetime(1). Despite comprehensive characterization of anxiety phenomenology, the underlying neural mechanisms—especially those involving rhythmic neural synchronization within sensory-emotional pathways—require further elucidation. Growing evidence indicates that the olfactory bulb (OB), while traditionally studied for its role in chemosensation, participates in broader neural processes beyond odor detection. Recent investigations have established that the OB processes both chemical and mechanical sensory information, with respiratory-driven air currents inducing characteristic neural oscillation patterns in local circuits (2, 3). The OB maintains extensive bidirectional connections with key limbic and cortical regions, including direct projections to the prefrontal cortex (PFC), amygdala, and hippocampus(4, 5). suggesting its potential involvement in emotional regulation. The PFC, known for its supervisory cognitive functions, coordinates higher-order processes including executive control, working memory maintenance, and emotional evaluation(6, 7). Its established participation in threat assessment and fear regulation (8-10). implies that OB-PFC

interactions may form a crucial neural substrate for anxiety pathology. The potential coordination between these structures via rhythmic neural synchronization represents a promising but underexplored area in affective neuroscience. Local field potentials (LFPs), generated by aggregated postsynaptic potentials within neuronal populations, provide crucial insights into collective neural activity. Modern electrophysiological research has revealed that LFPs encode both local circuit operations and distributed network communications(11). These extracellular voltage oscillations are typically divided into distinct frequency ranges: delta (1-4 Hz), theta (4-8 Hz), alpha (8-12 Hz), beta (13-30 Hz), and gamma (30-80 Hz) bands, each associated with specific cognitive and behavioral functions. While previous work has demonstrated enhanced theta-band synchronization between OB and PFC during anxious states(12). But high frequency gamma bands behavior in anxiety state remains unknown. Building upon this foundation, the current study examines high-frequency oscillatory coupling between OB and PFC circuits during anxiety states, focusing specifically on gamma-range synchronization patterns. This investigation aims to elucidate the potential role of fast rhythmic activity in sensory-prefrontal communication during threat processing.

Methods

Animal Subjects and Housing

The study utilized twelve adult male Wistar rats obtained from the Laboratory Animal Center of Zahedan University of Medical Sciences (Ethical Approval Code: IR.ZAUMS.REC.1399.443). Animals were housed under controlled environmental parameters, maintaining temperature at 21 ± 0.5°C and relative humidity at 50 ± 5%, with a 12-hour light-dark cycle (illumination period: 07:00-19:00). Standard laboratory chow and purified water were provided ad libitum throughout the study duration.

Surgical Procedures

All surgical operations were conducted under sterile conditions. Anesthesia was induced via intraperitoneal injection of ketamine HCl (100 mg/kg) combined with xylazine HCl (10 mg/kg) in physiological saline (0.9% NaCl). Surgical plane anesthesia was confirmed by absence of both pedal withdrawal and corneal reflexes prior to stereotaxic (Narishige, Japan) immobilization. Chronic electrode implantation targeted two neuroanatomical regions, Olfactory bulb (OB): +8.5 mm AP, -1.0 mm ML, -1.5 mm DV relative to bregma and Prefrontal cortex (PFC): +3.2 mm AP, -0.6 mm ML, -3.6 mm DV. Electrode arrays consisted of Teflon-coated stainless steel microwires (127 μm diameter, A.M. system Inc., USA). A stainless-steel reference screw (1 mm diameter) was affixed to the right parietal cortex (-3.0 mm AP, +2.0 mm ML). All electrodes were connected to a miniaturized 6-pin connector and permanently secured with dental acrylic. Postoperative care included topical tetracycline (10%) application and maintenance at 37°C during the 7-day recovery phase.

Electrophysiological Recording Protocol

Following recovery, simultaneous LFP recordings were obtained during behavioral sessions using a BIODAC-Bi401l9B system (TRITA Health Tec. Co., Tehran, Iran) with 2 kHz sampling rate. Acquired signals underwent hardware-based low-pass filtering (250 Hz cutoff, 4th-order Butterworth) before digitization. Signal quality was assessed through visual examination of raw waveforms (representative example in Fig. 1C). Behavioral monitoring employed a high-resolution camera (30 fps) mounted 1.5 m above the testing apparatus, temporally synchronized with neural recordings.

Behavioral Tests

Animals underwent 7-day habituation to the experimental environment before testing. Behavioral evaluation commenced on postoperative day 7, with subjects randomly assigned to either Elevated plus maze (EPM), 10-minute sessions in apparatus featuring two open arms (50×10 cm) and two enclosed arms (50×10 cm × 40 cm height), elevated 50 cm above floor level (Fig 1.A) or Open field (OF): 10-minute sessions in 50×50 cm arena with 50 cm high walls (Fig 1.B)

Histological Processing

Following behavioral testing, euthanasia was

performed via urethane overdose (1.2 g/kg intraperitoneally). Brains were rapidly extracted and fixed in 4% paraformaldehyde (in 0.1M phosphate buffer, pH 7.4) at 4°C for 48 hours. Coronal sections (50 µm) were obtained using a precision brain matrix.

Data Analysis

Neural oscillations were isolated using Butterworth bandpass filters. Functional connectivity was assessed via Granger causality (GC) analysis (Formula 1).

(Formula 1)

$$I_{X\to Y}(f) = \ln\left(\frac{S_{YY}(f)}{S_{YY}(f) - \left(\Sigma_{XX} - \frac{\Sigma_{XY}^2}{\Sigma_{YY}}\right)|H_{XY}(f)|^2}\right)$$

where X→Y indicates directional influence, S represents power spectra, H denotes transfer function, and Σ is noise covariance. GC computations utilized the MVGC toolbox (MATLAB 2016, The Mathworks Inc., USA) with model order 10. Statistical comparisons employed Wilcoxon signed-rank tests (significance threshold: $p<0.05$).

Results

To solidify results, rats performed experiments on two anxiety provoking apparatuses, elevated plus maze and open field (Fig 1.A, B).

Next, investigation of neural communication patterns revealed significant directional coupling between olfactory and prefrontal circuits. In the low gamma frequency range (30-50 Hz), Granger causality analysis demonstrated substantially stronger information flow from the olfactory bulb to prefrontal cortex (OB→PFC) compared to the reverse direction (PFC→OB). This effect was consistently observed across both behavioral assessments, reaching statistical significance in the elevated plus maze (EPM: $p=0.01$, Fig. 2) and open field (OF: $p=0.004$, Fig. 3) paradigms.

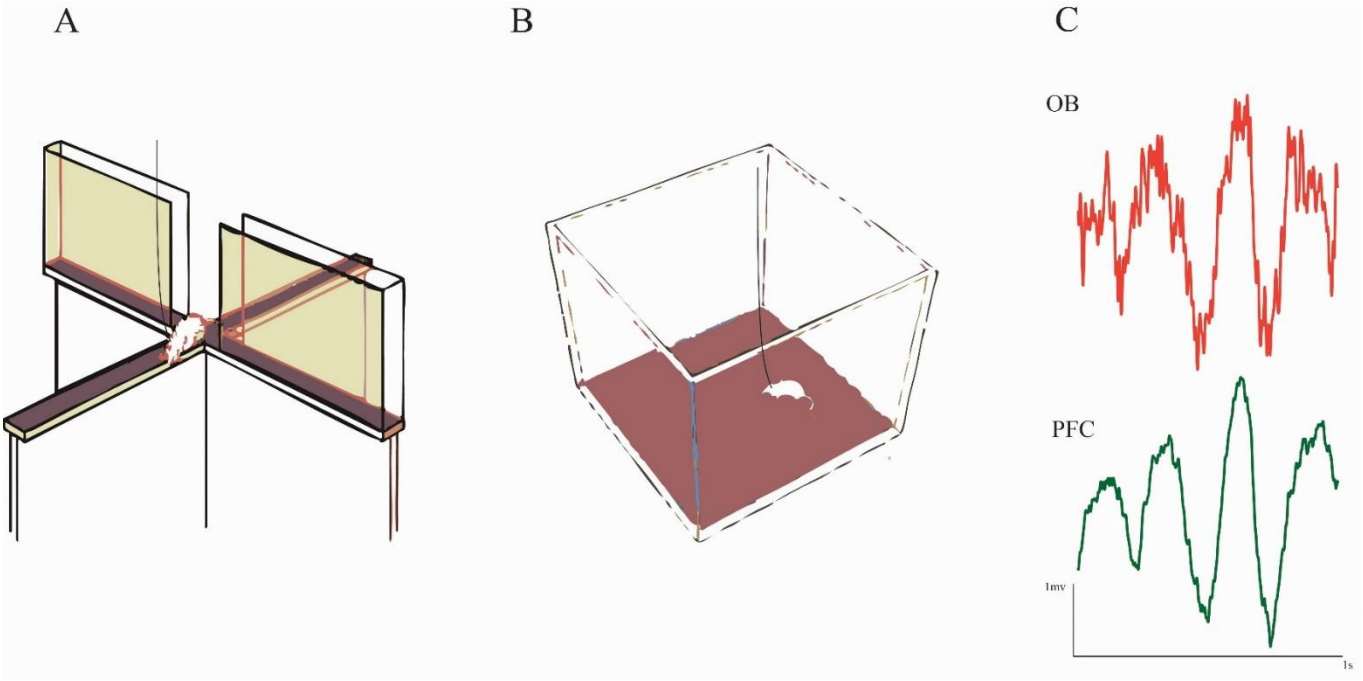

Fig 1. experimental devices and sample recording. A, B) elevated plus maze (A) and open field (B) devices. C)LFP recording sample of OB and PFC.

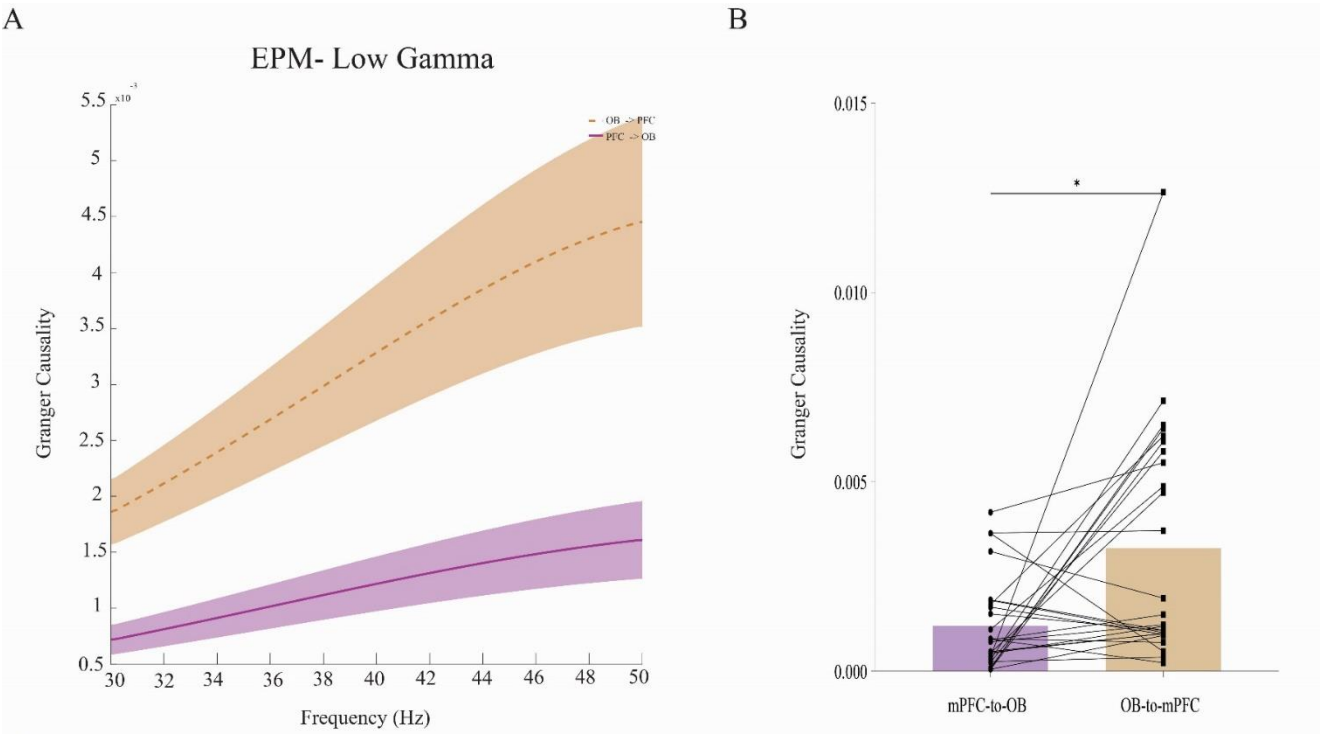

Fig 2. Low gamma granger causality in EPM. Spectrogram (Left panel) and mean of each trial comparison (Right panel) between low gamma granger causality in OB-to-PFC and PFC-to-OB pathways of EPM group.

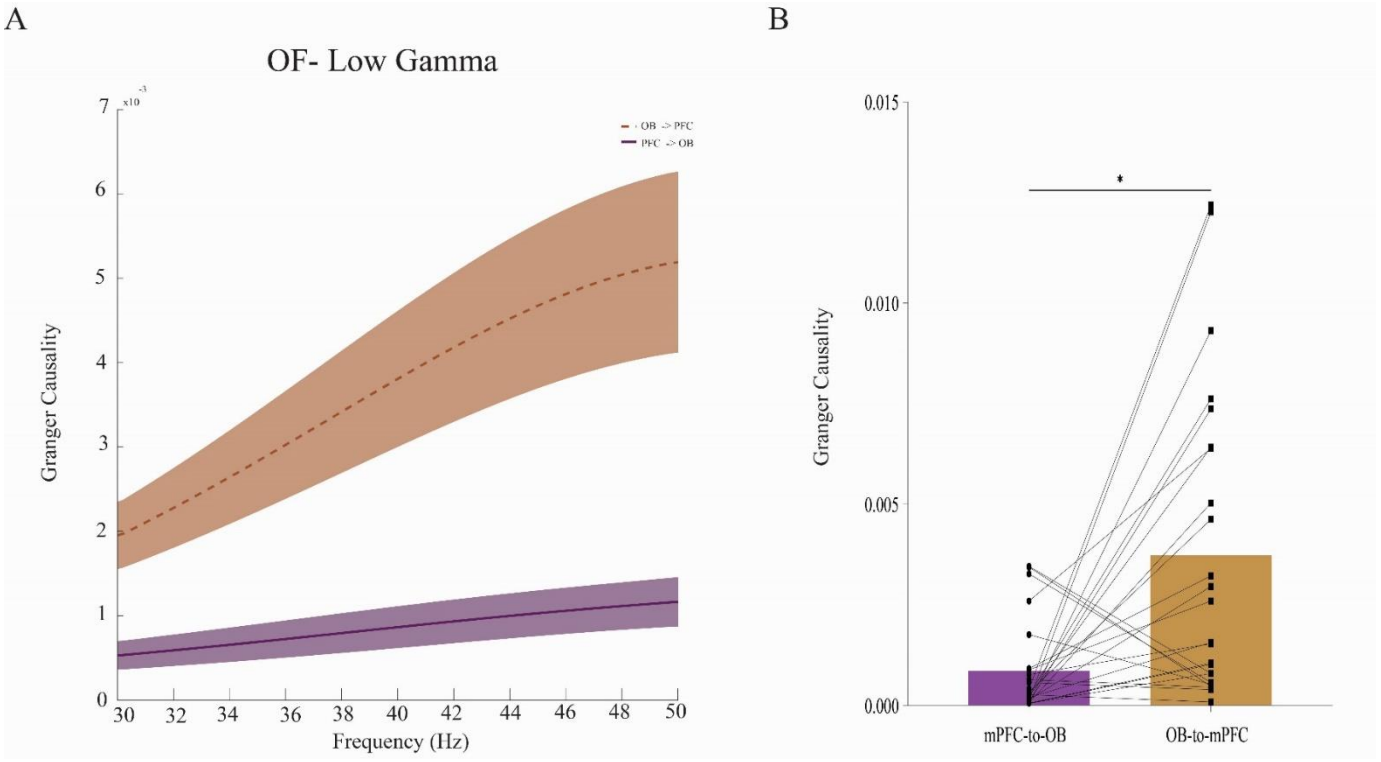

Fig 3. Low gamma granger causality in OF. Spectrogram (Left panel) and mean of each trial comparison (Right panel) between low gamma granger causality in OB-to-PFC and PFC-to-OB pathways of OF group.

The same directional preference was evident in high gamma oscillations (50-100 Hz), where OB→PFC connectivity measures significantly exceeded PFC→OB values in both experimental conditions (EPM: p=0.03, Fig. 4; OF: p=0.01, Fig. 5). The maintenance of this unidirectional pattern across both gamma sub-bands implies a fundamental organizational principle governing olfactory-prefrontal communication during anxiety states.

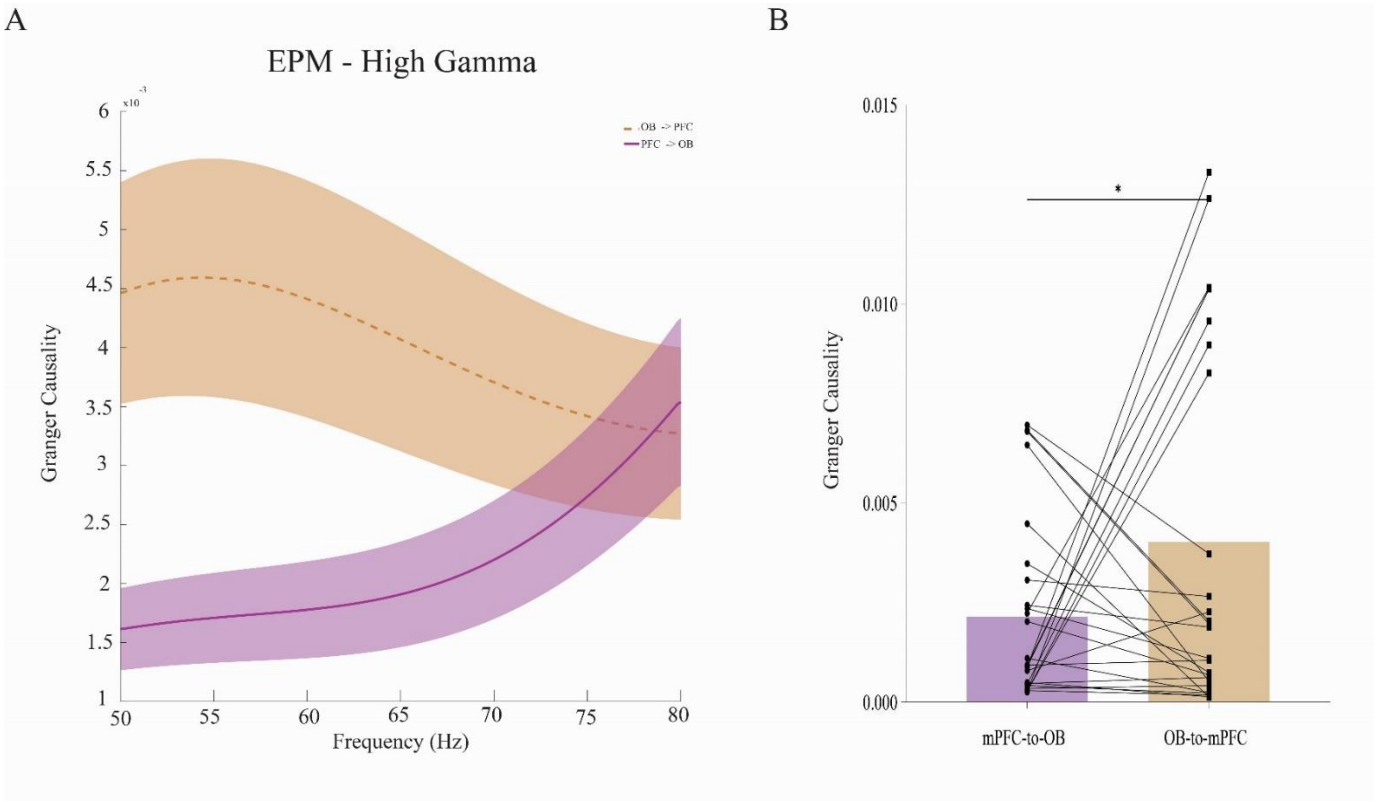

Fig 4. High gamma granger causality in EPM. Spectrogram (Left panel) and mean of each trial comparison (Right panel) between High gamma granger causality in OB-to-PFC and PFC-to-OB pathways of EPM group.

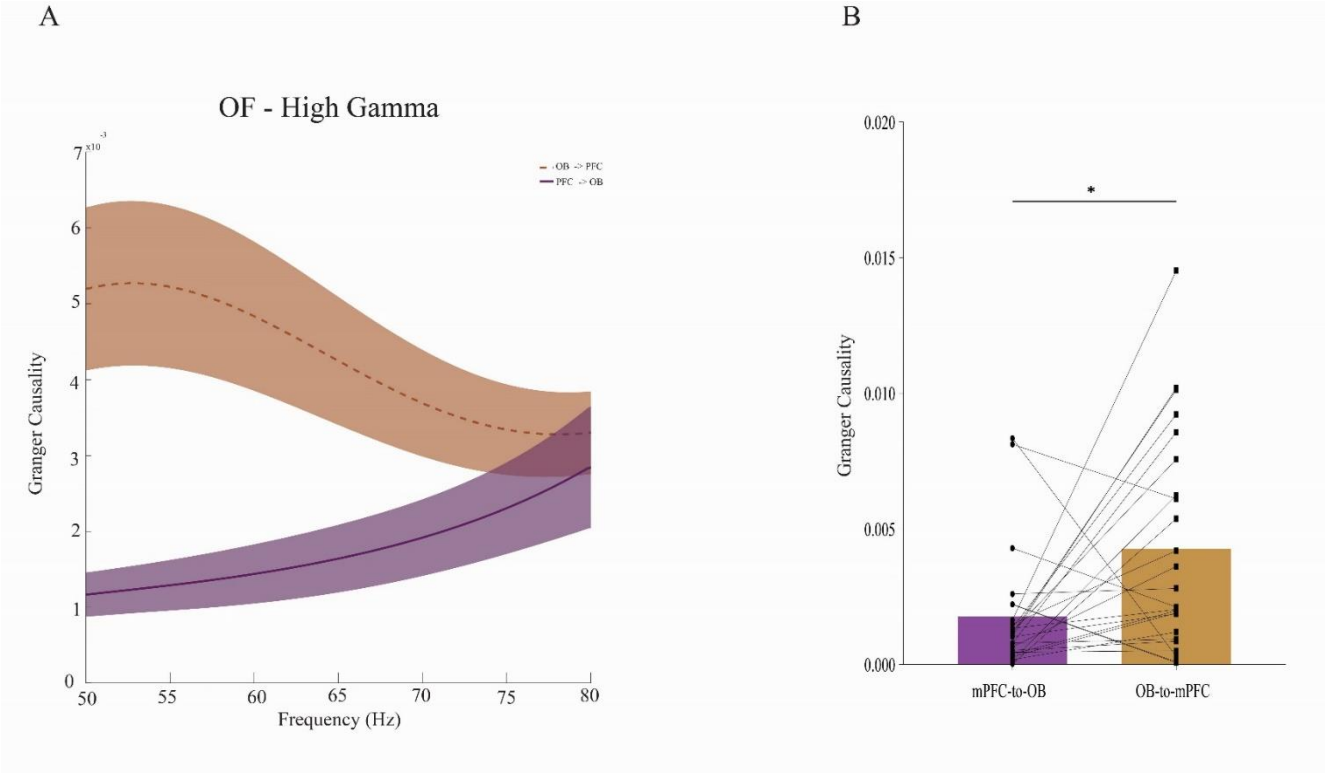

Fig 5. High gamma granger causality in OF. Spectrogram (Left panel) and mean of each trial comparison (Right panel) between High gamma granger causality in OB-to-PFC and PFC-to-OB pathways of OF group.

Discussion

This study offers new insights into the role of gamma-band oscillations in facilitating directional interactions between olfactory and prefrontal regions during anxiety. The prominent feedforward connectivity from the olfactory bulb

(OB) to the prefrontal cortex (PFC) observed in both low (30–50 Hz) and high (50–100 Hz) gamma frequencies indicates that rapid neural rhythms may be central to how sensory-limbic circuits process threat-related information. These results contribute to a deeper understanding of anxiety-related brain dynamics by implicating gamma synchrony in olfactory-prefrontal communication during heightened emotional states. The consistent pattern of OB→PFC information flow across two behavioral models of anxiety—elevated plus maze (EPM) and open field (OF)—reinforces the view that gamma-band coupling is a stable and biologically meaningful feature of anxiety circuitry (EPM: low gamma $p = 0.01$, high gamma $p = 0.03$; OF: low gamma $p = 0.004$, high gamma $p = 0.01$). Rather than reflecting task-specific phenomena, this directional coupling appears to reflect a broader organizational principle. These findings are in line with current models suggesting that gamma oscillations support the integration of sensory input and higher-order processing(13), and extend such models to include olfactory-prefrontal pathways. The greater granger causality observed in the low gamma range may correspond to its hypothesized role in coordinating activity across brain regions, whereas high gamma may underpin more localized computations within target areas(14). Several neural mechanisms may be responsible for the observed gamma synchronization. The olfactory bulb's dual function as a sensory processor and rhythm generator may enable it to shape prefrontal dynamics during threat evaluation(2, 3). Additionally, the PFC's known involvement in appraising emotionally salient stimuli may enhance its receptivity to rhythmic input from olfactory structures(8-10). Reciprocal anatomical pathways between OB and PFC could further promote synchronization through bidirectional resonance in the gamma range(4, 5). These findings carry several theoretical implications. First, I suggest that anxiety-related integration across sensory and limbic areas may rely on precisely timed gamma activity, complementing the well-characterized role of theta oscillations in affective processing. Second, the unidirectional nature of connectivity points to a potentially causal influence of olfactory inputs on prefrontal function during anxiety. Lastly, the replication across distinct behavioral paradigms supports the idea that this gamma-mediated communication represents a general mechanism underlying anxiety, rather than one limited to specific contexts or stimuli.

Conclusion

My findings reveal that gamma-band activity supports strong feedforward signaling from olfactory to prefrontal regions during anxiety-related states. The reproducibility of this directional connectivity across distinct gamma frequency bands and behavioral models suggests it may serve as a core mechanism underlying sensory-limbic coordination in threat-related contexts. These results offer new perspectives on the neural basis of anxiety and point to gamma synchrony within the OB–PFC pathway as a potential focus for therapeutic strategies. By highlighting frequency-specific patterns of directional interaction, this work provides a novel framework for understanding how sensory information modulates cognitive and emotional processing in anxiety disorders.

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Conflict of interest

The author declare that have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Authors contribution

AS: conducting the experiment; data analysis; writing, and editing the

Manuscript.

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