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Anxiety-like States Enhance Beta-Band Functional Connectivity Between the Olfactory Bulb and Prefrontal Cortex

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ABSTRACT

Introduction: Anxiety disorders involve altered brain circuit dynamics, but the neural mechanisms underlying olfactory-prefrontal interactions during anxiety remain unclear. Beta oscillations are implicated in long-range neural communication and emotional processing, but their specific role in anxiety remains underexplored.

Methods: I recorded simultaneous local field potentials (LFPs) from the olfactory bulb (OB) and prefrontal cortex (PFC) in male Wistar rats during anxiety-provoking behavioral tests, including the Elevated Plus Maze (EPM) and Open Field (OF). Functional connectivity was assessed through beta-band (13–30 Hz) synchronization using cross-correlation and power correlation analyses.

Results: Behavioral tests revealed a significant increase in beta-frequency synchronization between OB and PFC during anxiogenic conditions compared to resting states. Notably, enhanced functional coupling was observed specifically in anxiogenic zones—the open arms of the EPM and the center area of the OF. These effects suggest an intensity-dependent increase in OB-PFC interaction associated with anxiety states.

Conclusion: My findings identify beta-band OB-PFC synchronization as a novel electrophysiological marker of anxiety, providing insight into sensory-executive integration during threat processing. This enhanced connectivity may contribute to the neural circuitry underlying anxiety and represents a potential target for therapeutic intervention.

Keywords:

Beta oscillations, Correlation, LFP.

1. Introduction

Anxiety is a state with perceptions of threat in the absence of real harmful stimuli, which has negative effects on the cognitive and behavioral functioning of the individual. As one of the commonest mental disorders, anxiety disorders have a severe psychological burden in the affected individuals as well as socioeconomic costs. On the basis of epidemiological research, it is estimated that about 30% of the world population develop clinically significant symptoms of anxiety at some point in their lives. (1, 2).Although the clinical relevance of anxiety is well-established, the precise neural mechanisms underlying its pathogenesis remain elusive, highlighting the need for further investigation in this field. The olfactory bulb (OB), located in the anterior part of the brain, plays a crucial role beyond olfactory processing. This region receives mechanosensory signals from nasal cavity receptors, generated by flow of air, and produces oscillatory activity (3, 4). Recent findings suggest that the olfactory system contributes to emotional processing and is implicated in stress-related disorders, including anxiety (5-9). Given the OB's extensive connections with key brain regions involved in cognitive processes such as the prefrontal cortex (PFC), amygdala, and hippocampus it may contribute to anxiety

regulation(10-12). The PFC, in particular, supports higher-order cognitive processes such as decision-making, memory, and the modulation of emotional states (13-16). Previous studies have demonstrated its essential role in anxiety- and fear-related processes (17-22). Given the integrative functions of both the OB and PFC, their dynamic interaction may represent a substrate for the modulation of anxiety; however, requires further investigation. Local field potentials (LFP) is key indicator of neuronal population activity, reflecting the summation of electrical currents generated by neurons in a specific brain region. Recent studies suggest that LFPs not only represent local neural activity but may also encode long-range functional connectivity between distant brain areas (23, 24). These oscillations are classified into distinct frequency bands: delta (<4 Hz), theta (4–8 Hz), alpha (8–12 Hz), beta (13–30 Hz), and gamma (30–120 Hz), each associated with specific cognitive functions. Among these, beta oscillations (13–30 Hz) have emerged as a particularly salient marker of cognitive states, being associated with wakefulness, sustained attention, and motor control. Within the OB, beta oscillations are notably enhanced during olfactory learning and are involved in the encoding and transfer of learned associations, as evidenced by increased beta-band coherence between the OB and hippocampus during odor-reward learning (25, 26). Given the involvement of beta oscillations in large-scale neural communication and their established role in cognitive processing, it is possible that they contribute to the neural mechanisms underlying anxiety. Based on the above evidence, this study investigates alterations in beta oscillations in the OB and PFC during anxiety states.

2. Materials & Methods

2.1. Animals

Twelve male Wistar rats were obtained from the Laboratory Animal Center of Zahedan University of Medical Sciences. The animals were housed under standard conditions (temperature: 21 ± 2°C; 12:12 light-dark cycle) with ad libitum access to food and water. All procedures were confirmed by the "Ethics Committee of Zahedan University of Medical Sciences" (IR.ZAUMS.REC.1399.443).

2.2. Surgery and Electrophysiological Recording

Animals were anesthetized using an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). After ensuring deep anesthesia, they were mounted on a stereotaxic apparatus (Narishige, Japan). Teflon-coated Stainless-steel electrodes (diameter: 203 μm, A.M. system Inc., USA) were implanted for local field potential recording in the olfactory bulb (OB; AP: +8.5 mm, ML: −1 mm, DV: −1.5 mm) and prefrontal cortex (PFC; AP: +3.2 mm, ML: −0.6 mm, DV: −3.6 mm). A stainless-steel screw was fixed in the right parietal bone as a reference electrode. Electrodes were integrated into a socket and secured to the skull with dental cement. The surgical area was treated with tetracycline ointment, and animals were allowed to recover for one week. Post-recovery, simultaneous LFP recordings from the OB and PFC were performed during behavioral tests using a BIODAC-Bi401l9B system (TRITA Health Tec. Co., Tehran, Iran). Signals were sampled at 2 kHz with a 250 Hz low-pass filter. To prove quality of recorded signal, Sample of raw signal presents in Fig 1.B. Animal behavior was concurrently recorded via a ceiling-mounted camera. After behavioral testing, rats were euthanized via urethane overdose (1.2 g/kg), and brains were extracted for electrode placement verification. Brains were fixed in 4% formaldehyde for 48 hours and sectioned using a brain matrix.

2.3. Behavioral Tests

To acclimate to the environment, animals were placed in the recording room one week before and after surgery. On days 7 and 8 post-surgery, behavioral tests were conducted (Fig 1.A). On day 7, LFP recordings from the OB and PFC were performed in home cage during resting state. On day 8, six rats underwent the elevated

plus maze (EPM) test, while the remaining six were tested in the open field (OF). The EPM test consisted of a 10-minute session on a plus-shaped maze with two open arms (50 × 10 cm) and two enclosed arms (50 × 10 cm, elevated 50 cm above the floor). The OF test involved a 10-minute session in a 50 × 50 cm square arena with 50 cm-high walls. Anxiety-inducing zones were defined as the open arms in the EPM and the central 30 × 30 cm area in the OF, while safe zones included the enclosed arms in the EPM and the peripheral areas in the OF. Trials for analysis considered as every movement from open to closed arms or reverse move.

2.4. Data Analysis

Beta oscillations were extracted from raw LFP data using a 13–30 Hz Butterworth bandpass filter. Power spectral density was calculated using Fourier transform, and Pearson correlation was computed between OB and PFC power values (Formula 1).

(1)
$$\rho_{X,Y} = \frac{\sum_{i=1}^{n}(X_i - \bar{X})(Y_i - \bar{Y})}{\sqrt{\sum_{i=1}^{n}(X_i - \bar{X})^2 \sum_{i=1}^{n}(Y_i - \bar{Y})^2}}$$

Values X and Y shows as power values of OB and PFC respectively.

Cross-correlation analysis was performed using a normalized time-lagged correlation (Formulas 2–3).

(2)
$$\rho_{xy}[m] = \frac{R_{xy}[m]}{\sqrt{R_{xx}[0] \cdot R_{yy}\left[\cdot\right]}}$$

(3)
$$R_{xy}[m] = \sum_{n=-\infty}^{\infty} x[n] \cdot y^*[n-m]$$

Values R_{xy} shows cross correlation between x and y signal. Value m represents time lag. The following MATLAB functions were used for data processing: butter, filtfilt for band-pass filtering; fft for spectral analysis; xcorr for cross-correlation; and custom scripts using corrcoef for power correlation.

For statistical comparisons, the Mann-Whitney U test was used to compare rest and behavioral test conditions within each group, while the Wilcoxon signed-rank test was employed for intergroup comparisons. All analyses were conducted in MATLAB v2016 (The Mathworks Inc., USA), with a p-value < 0.05 considered statistically significant.

3. Results

Initial comparisons were made between resting state and behavioral test conditions in both experimental groups (Protocols describe in Fig 1.A).

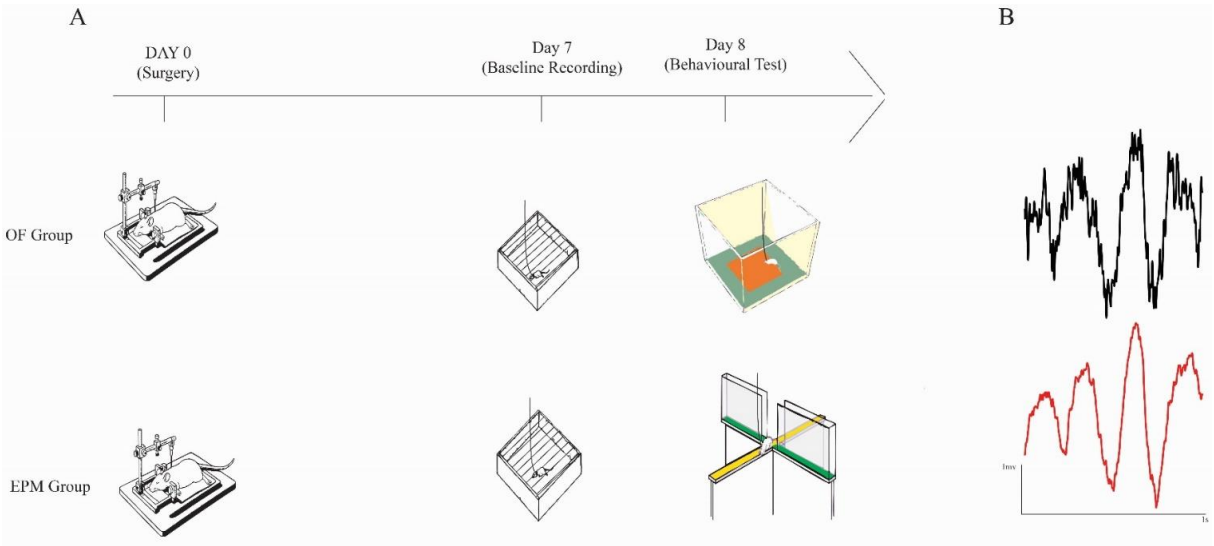

Fig 1. Schematic representation of experiments.
A) All animals went through electrode implementation surgery at day 0 and baseline recording at day7.Next, they perform behavioral test at day 8. B) Sample of OB and PFC raw LFP signal.

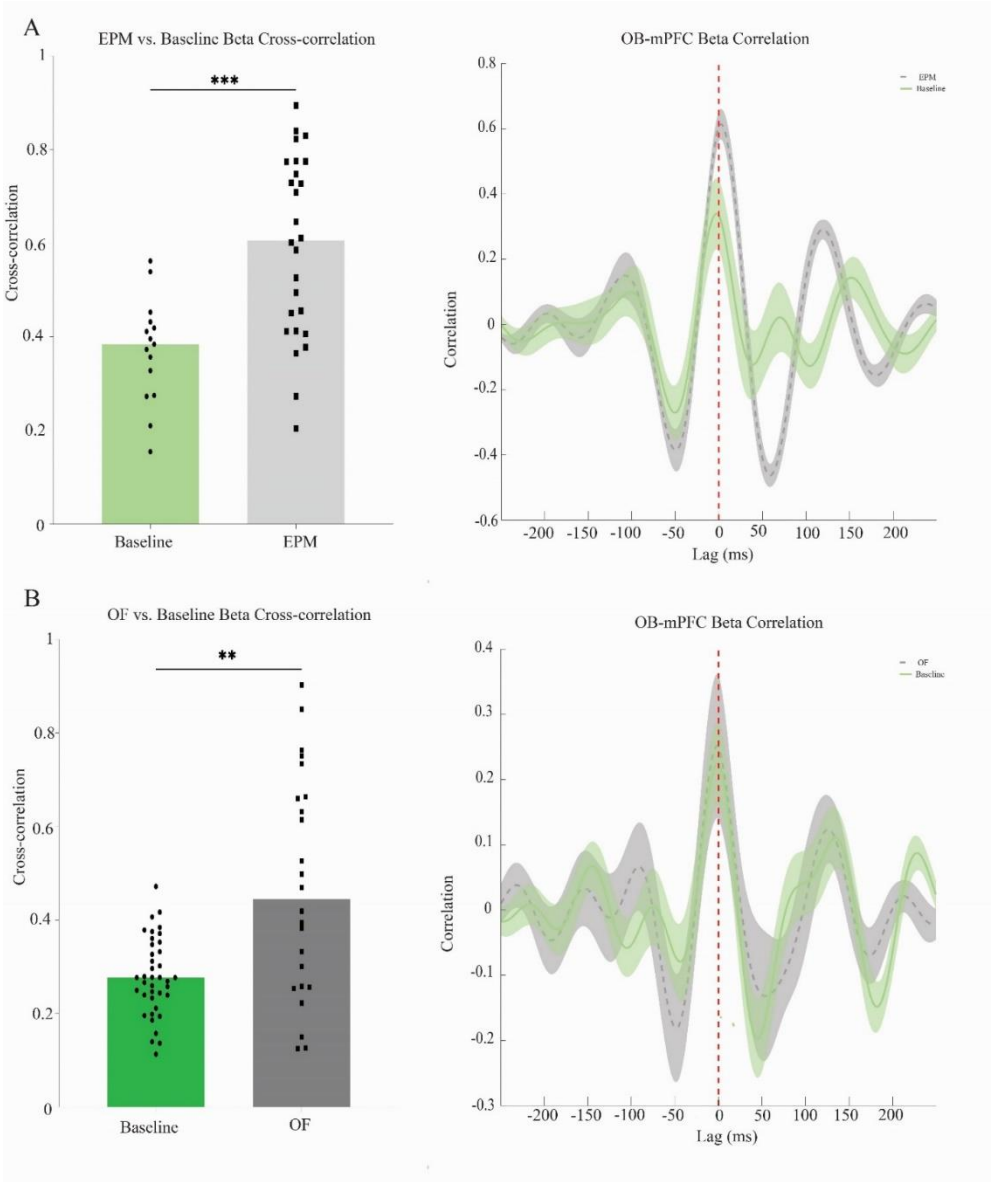

Fig 2. OB-PFC Cross-correlation in baseline vs. behavioral test. OB-PFC Beta cross-correlation (Right panel) and peak values (Left panel) of baseline session and test session in EPM (A) and OF(B) group (each point in figures represents one trial).

In the EPM group, cross-correlation analysis representing functional connectivity between the two regions revealed significantly increased beta-band synchronization between olfactory bulb (OB) and prefrontal cortex (PFC) oscillations during behavioral testing compared to resting state (p-val = 0.0004; Fig 2.A). Similarly, in the OF group, cross-correlation between OB and PFC oscillations showed significant enhancement in the beta frequency range during central and peripheral trials relative to baseline (p-val = 0.0015; Fig 2.A).

These findings demonstrate strengthened functional coupling between OB and PFC during anxiety-provoking behavioral tests (EPM and OF) compared to resting state. I further examined power correlation between the two regions as another measure of functional connectivity. During EPM testing, OB and PFC oscillation power showed significantly positive correlation (r= 0.34, p-value=0.04; Fig 3.A). The OF test similarly demonstrated positive power correlation between these regions (r= 0.36, p-value=0.02; Fig 3.B), providing additional evidence for enhanced OB-PFC communication during anxiety states.

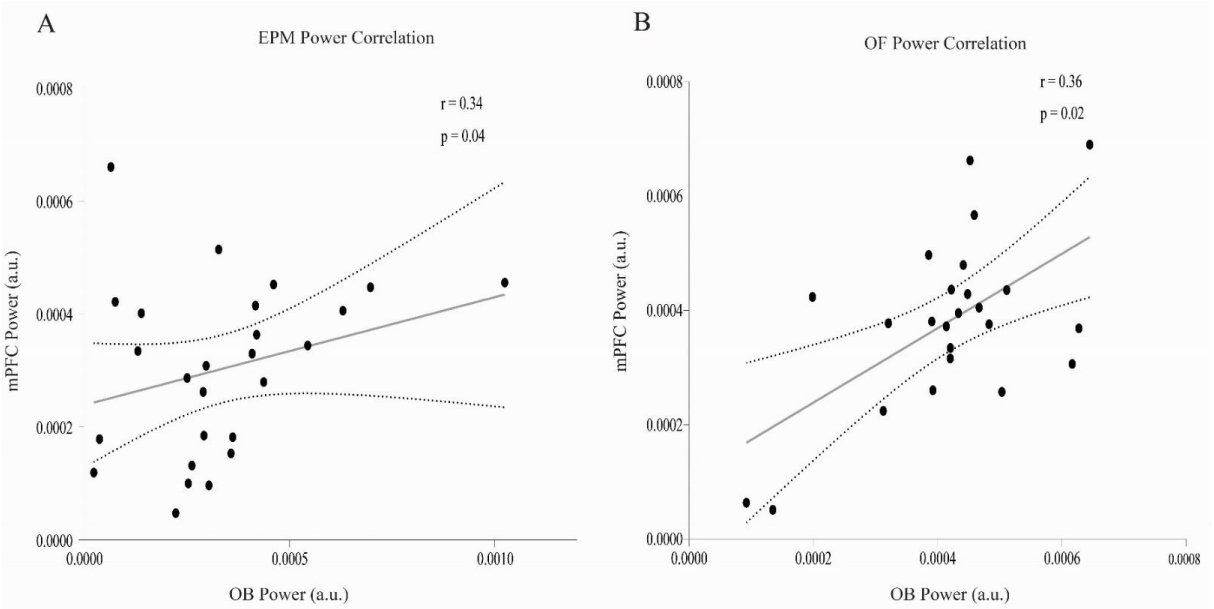

Fig 3. OB-PFC power correlation in behavioral tests. OB-PFC Beta power correlation in EPM (A) and OF(B) group (each point in figures represents one trial).

To validate these findings, I compared anxiety-provoking versus safe zones within each behavioral test. In the EPM group, beta-band cross-correlation between OB and PFC was significantly higher when animals explored open arms compared to enclosed arms (p-value=0.04; Fig 4.A). Correspondingly, in the OF group, central area exploration (anxiogenic zone) produced markedly greater OB-PFC cross-correlation than peripheral area movement (safe zone) (p-value=0.004; Fig 4.B).

Collectively, these results demonstrate that anxiety states in male rats are associated with enhanced functional connectivity between beta-band oscillations in the OB and PFC, suggesting these structures may participate in a coordinated network during anxiety responses.

4. Discussion

In this study, I examined the functional connectivity between the olfactory bulb (OB) and the prefrontal cortex (PFC) during anxiety states, using simultaneous LFP recordings in rats. In the context of anxiety, a previous study has shown modulation of beta activity in the PFC during stress exposure(27). But, Another study reports that frontal beta oscillations increases during anxiety state(28).About OB and PFC interaction, studies mainly reports theta and gamma interaction (9, 29). But, Betha rhythms remain elusive in anxiety state. In this way, my findings revealed a clear enhancement beta-band (13-30 Hz) synchronization between these regions during anxiety-provoking behavioral tests (EPM and OF) compared to resting state. This pattern of increased beta synchrony, observed through both cross-correlation and power correlation analyses, provides novel electrophysiological evidence implicating OB–PFC interactions in the neural processing of anxiety. The observed enhancement of beta-band correlation between OB and PFC activity during anxiety tests aligned with several lines of existing evidence. First, Both regions, individually, have well-documented roles in emotional regulation and stress responsiveness (9, 30). Second, beta oscillations are known to mediate long-range communication between distant brain regions during cognitive tasks (31-34). In the present study, the most robust increases in OB–PFC coupling emerged within the most anxiogenic zones of the behavioral paradigms (open arms of EPM and center of OF), suggesting a direct relationship between anxiety intensity and OB-PFC coupling strength. The specific involvement of beta-band oscillations may reflect their role in maintaining heightened

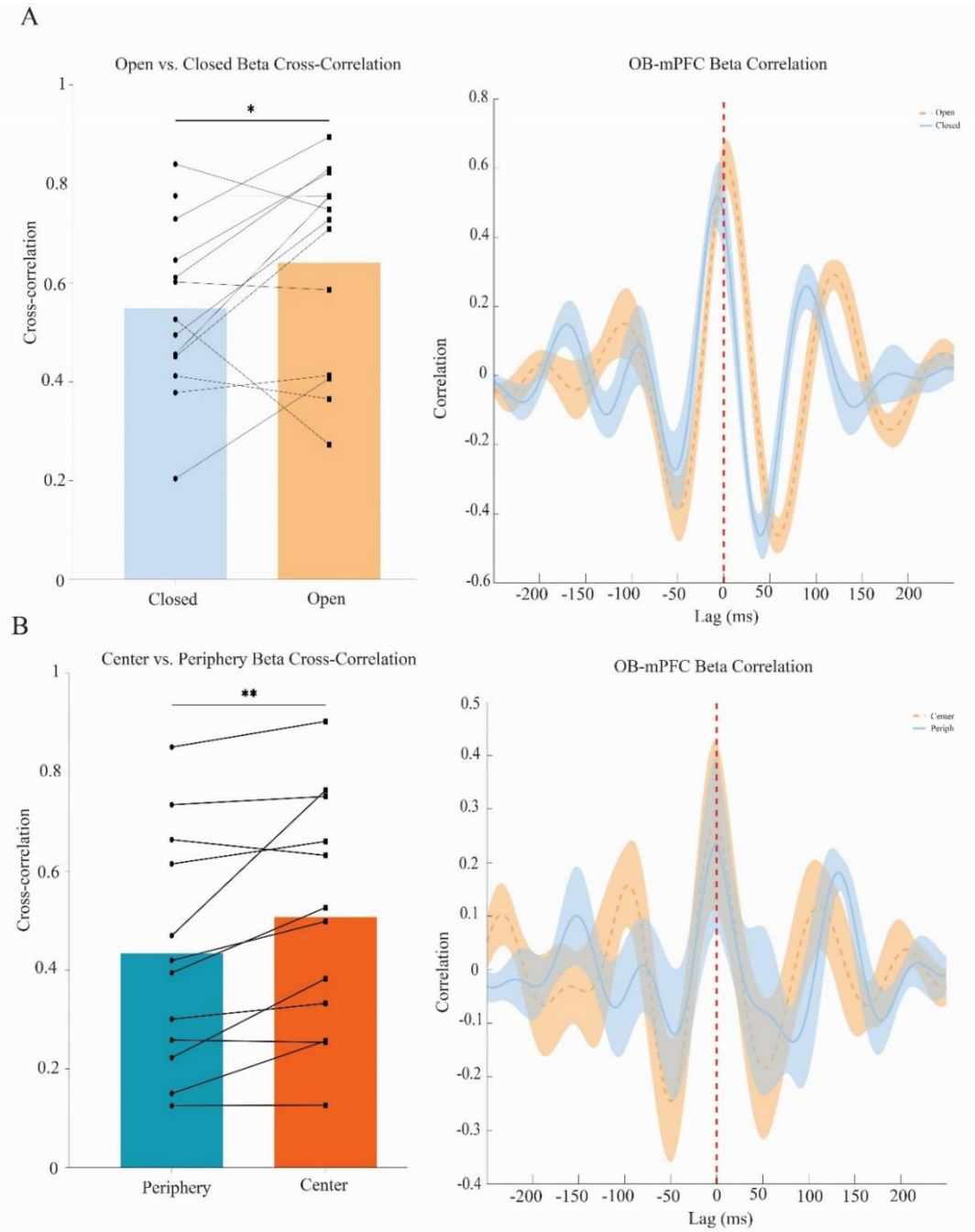

Fig 4. OB-PFC Cross-correlation between anxiogenic and safe zone. OB-PFC Beta cross-correlation (Right panel) and peak values (Left panel) of anxiogenic zone and safezone in EPM (A) and OF(B) group (each point in figures represents one trial).

vigilance and attention during threatening situations. My findings extend previous work showing beta synchronization during olfactory learning to the domain of emotional processing(25, 26). I proposed that enhanced OB-PFC beta correlation represents a mechanism for integrating sensory (potentially olfactory) information with higher-order executive functions. Such integration could guide adaptive behavioral responses in the presence of danger. Future studies should examine these findings in human subjects using non-invasive techniques and investigate causal relationships through manipulation of the OB-PFC circuit.

5. Conclusion

this study offered new evidence that beta-band synchronization between the olfactory bulb and prefrontal cortex increases during anxiety-like states in rats.This behavior likely reflects

an interplay between sensory processing and executive control when threatening perceived. These findings may contribute to a better understanding of how the brain integrates sensory information with emotional processing during anxiety. While further research is needed to confirm these patterns and clarify the causal role of OB-PFC coupling in anxiety behavior.

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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 experimental study; data analysis, writing, and editing the Manuscript.

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