



## Adaptive closed-loop modulation of cortical theta oscillations: Insights into the neural dynamics of navigational decision-making

Farhad Farkhondeh Tale Navi<sup>a</sup>, Soomaayeh Heysieattalab<sup>a</sup>, Mohammad Reza Raoufy<sup>b,\*\*</sup>, Saied Sabaghypour<sup>a</sup>, Milad Nazari<sup>c</sup>, Mohammad Ali Nazari<sup>a,d,\*</sup>

<sup>a</sup> Department of Cognitive Neuroscience, Faculty of Education and Psychology, University of Tabriz, Tabriz, Iran

<sup>b</sup> Department of Physiology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

<sup>c</sup> Department of Molecular Biology and Genetics, Aarhus University, Denmark

<sup>d</sup> Department of Neuroscience, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran

### ARTICLE INFO

#### Keywords:

Closed-loop neurofeedback (CLNF)  
Theta oscillations  
mPFC-vHPC functional connectivity  
SWM task  
Navigational decision-making

### ABSTRACT

Navigational decision-making tasks, such as spatial working memory (SWM), rely highly on information integration from several cortical and sub-cortical regions. Performance in SWM tasks is associated with theta rhythm, including low-frequency oscillations related to movement and memory. The interaction of the ventral hippocampus (vHPC) and medial prefrontal cortex (mPFC), reflected in theta synchrony, is essential in various steps of information processing during SWM. We used a closed-loop neurofeedback (CLNF) system to upregulate theta power in the mPFC and investigate its effects on circuit dynamics and behavior in animal models. Specifically, we hypothesized that enhancing the power of the theta rhythm in the mPFC might improve SWM performance. Animals were divided into three groups: closed-loop (CL), random-loop (RL), and OFF (without stimulation). We recorded local field potential (LFP) in the mPFC while electrical reward stimulation contingent on cortical theta activity was delivered to the lateral hypothalamus (LH), which is considered one of the central reward-associated regions. We also recorded LFP in the vHPC to evaluate the related subcortical neural changes. Results revealed a sustained increase in the theta power in both mPFC and vHPC for the CL group. Our analysis also revealed an increase in mPFC-vHPC synchronization in the theta range over the stimulation sessions in the CL group, as measured by coherence and cross-correlation in the theta frequency band. The reinforcement of this circuit improved spatial decision-making performance in the subsequent behavioral results. Our findings provide direct evidence of the relationship between specific theta upregulation and SWM performance and suggest that theta oscillations are integral to cognitive processes. Overall, this study highlights the potential of adaptive CLNF systems in investigating neural dynamics in various brain circuits.

### 1. Introduction

Navigational decision-making is considered an internal deliberation function that requires integrating immediate past information with a predetermined rule [1]. The maintenance of external world memory has been referred to as cognitive maps and is a critical part of spatial working memory (SWM) tasks [2]. This highly demanding task employs several functions, including spatial working memory [3], attention [4, 5], and motor behaviors [6] in spatial configurations such as T-mazes. SWM consists of several steps, including initial memory formation of the environment, its successive recall, and evaluation of options. It is

reported that the integrative process that occurs between several brain areas to support these functions is primarily supported by theta oscillations ([7] Wilson, 2005). These oscillations have been considered to have distinct contributions to bottom-up (sensorimotor-driven) and top-down (cognitive control) processing in reentrant cortical and subcortical communications (Ekstrom & Watrous, 2014).

Theta oscillations in the medial prefrontal cortex (mPFC) underpin a cognitive mechanism that preserves choice information and supports vicarious memory recall of several choices [8]. However, hippocampus (HPC) theta oscillations provide representational information regarding spatial paths during active navigation [9,10]. Theta oscillations also play

\* Corresponding author. Department of Neuroscience, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran.

\*\* Corresponding author.

E-mail addresses: [raoufy@modares.ac.ir](mailto:raoufy@modares.ac.ir) (M.R. Raoufy), [nazaripsycho@yahoo.com](mailto:nazaripsycho@yahoo.com) (M.A. Nazari).

<https://doi.org/10.1016/j.brs.2024.09.005>

Received 21 October 2023; Received in revised form 4 August 2024; Accepted 10 September 2024

Available online 12 September 2024

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essential roles in synchronizing and activating specific mPFC and HPC neural ensembles in SWM tasks 11–15. Specifically, this synchronization in animal research is mainly observed when the mPFC is modulated by hippocampal theta oscillations [16]. This phase-locked increase in PFC activity that regulates attention and decision-making (Menon & D'Esposito, 2022; [5]) might reflect the inputs of spatial-related information from the HPC that is critical for encoding location and navigation (Bird & Burgess, 2008). Despite substantial correlation evidence, there is a lack of research investigating the decisive roles and circuit underpinnings of the related oscillations in navigational tasks *in vivo* due to technological constraints.

The development of new closed-loop systems is expected to guide perturbations of neural systems to achieve sophisticated, real-time control over neural dynamics and animal behavior [17]. In particular, observing and feeding back the effects of circuit interventions on physiologically relevant timescales is valuable for directly testing inferred models of dynamics, connectivity, and causation (Ros, J. Baars, Lanius, & Vuilleumier, 2014). Closed-loop neurofeedback (CLNF) uses electrical stimulation to reward-associated regions of the brain. Incorporating both neurofeedback and intracranial self-stimulation (ICSS) [18,19] paradigms, such a system applies direct and contingent stimulation to reward-associated regions such as the lateral hypothalamus (LH) [20]. Recent research reveals that high-frequency LH stimulation, deactivating orexin neurons akin to eating behaviors [21,22], offers potential as a substitute for feeding in instrumental conditioning [23]. Similar animal studies have reported that Deep Brain Stimulation (DBS) of the fornix and medial septal nucleus can improve SWM and enhance hippocampal theta oscillations ([24]; Lee et al., 2012; [25]). In addition, the LH stimulation enhances relational learning and hippocampus-driven memory formation [26]. CLNF might regulate the neural properties of the circuit in such a way as to reach the desired variation in brain oscillations. The reward stimulation might indirectly affect the underlying circuit of a cortical rhythm and results in global modulation of the cortical and subcortical regions [27].

Here, we utilized an *in vivo* CLNF system within the ICSS paradigm to specifically enhance theta activity in mPFC (i.e., Theta-CLNF). We also investigated the subcortical changes occurring in vHPC and the relationship between the theta oscillations and spatial working memory after applying the Theta-CLNF protocol. The delayed non-match-to-sample (DNMS) test of SWM was conducted to evaluate the behavioral performance of animals. We hypothesized that enhancing the power of theta oscillations within the mPFC driven by LH stimulation would yield an increase in mPFC theta and mPFC-vHPC functional connectivity. Regarding the behavioral outcomes, we also expected an increase in the rate of correct responses and a reduction in the latency required to reach the correct arm during the SWM task. Exploring the potential influences of CLNF offers a promising avenue for modulating neural oscillations, shedding light on the intricate relationship between brain rhythms and cognitive processes.

## 2. Materials and methods

### 2.1. Animals and ethical statement

Twenty-one matured male Wistar rats weighing 240–280 gr, 10–12 weeks old, were obtained from the Razi Institute (Karaj, Iran). The animals were housed in  $21 \pm 2$  °C, 12-h light-dark cycle (light on 7:00–19:00) with unrestricted access to water and food during recovery period and feedback sessions. All experimental procedures and animal protocols were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (2011) and approved by the University/Regional Research Ethics Committee of the University of Tabriz (IR.TABRIZU.REC.1399.058).

### 2.2. Study design

Our study consisted of feedback (Fig. 1a) and behavioral (Fig. 1b) sessions. After one week of recovery, the local field potential (LFP) recordings were carried out for all groups. Every feedback session consisted of three steps: a 10-min pre-training, a 30-min training, and a 10-min post-training. Two open field tests were conducted at the beginning of the 1st and end of the 10th day of feedback sessions. Animals were divided randomly into three groups, including OFF (without stimulation), closed-loop (CL), and random-loop (RL) groups (each group contained seven animals) (Fig. 1c). This study design aligns with recent methodologies [28,29], incorporating distinct groups and comparing closed-loop stimulation with non-contingent and without stimulation controls.

### 2.3. Electrode implantation and histological verification

#### 2.3.1. Surgical procedures

Animals were anesthetized with intraperitoneal injections of ketamine (100 mg/kg) and xylazine (10 mg/kg). Anesthesia was confirmed by the lack of paw withdrawal, whisker movement, and eye blink reflexes, and the animal was put on a heating pad with an anal probe to keep the body temperature at approximately 37.5 °C. A stereotaxic apparatus was used for the fixation of anesthetized rats. Prior to the surgery, 0.5 ml of lidocaine chlorhydrate 2 % was subcutaneously injected into the scalp for local anesthesia. Then, a longitudinal mid-sagittal incision was made on the scalp to expose the skull. For LFP recording, stainless-steel electrodes (127 µm in diameter, A.M. System Inc., USA) were implanted unilaterally into stereotaxic coordinates of left mPFC (AP +3.2 mm; L –0.6 mm; DV –3.6 mm) and vHPC (AP –5.6 mm; L –5.5 mm; DV –7.4 mm) according to the rat brain atlas [30]. For reward stimulation, one twisted tungsten bipolar electrode was placed into the ipsilateral LH, one of the reward-related regions of the brain (AP –2.8 mm; L –1.7 mm; DV –8.5 mm) (Fig. 1d). Two additional holes in the skulls were used to hold the recording socket. A stainless-steel screw was implanted at the right side of the parietal bone as a reference point. The acrylic dental adhesive was poured around the electrodes and bone screw. For the free-moving method, after mounting reference screws and anchoring screws, dental cement was applied, and a plastic pedestal was fixed on top of the rat's head, with sockets of implanted electrodes placed inside. The skin was sutured, and an antibiotic ointment was used for the wound to prevent infection.

#### 2.3.2. Histological verification

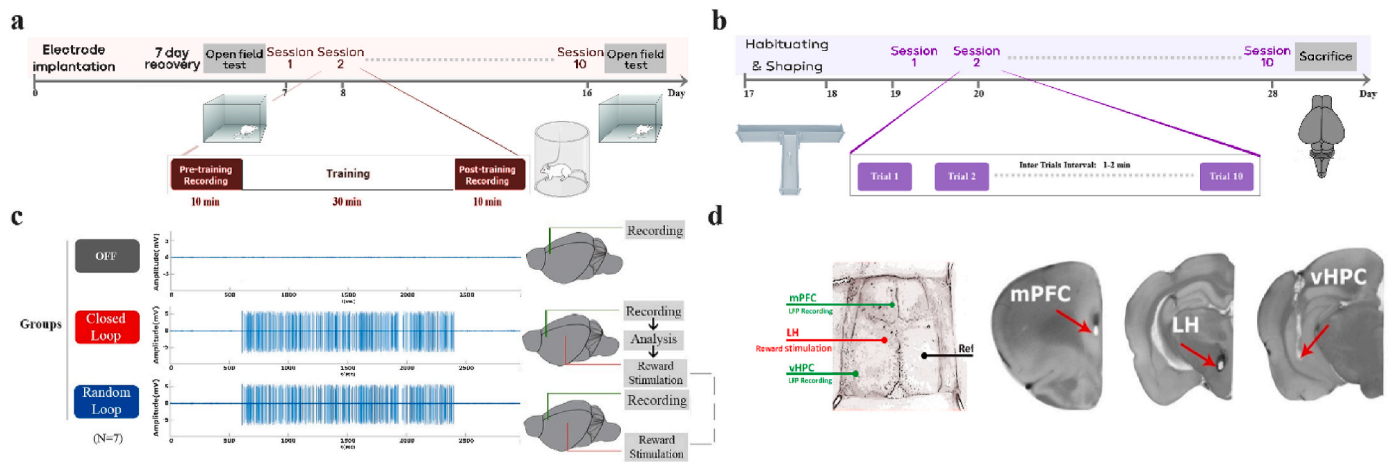
Histology of the implanted electrodes was made by current injection after the experiments. Animals were deeply anesthetized, and electrolytic lesions were made in the stimulation and recording electrodes to determine the position of the electrode tip (Benz, 2016).

To confirm that the electrodes were implanted in the desired location, the rats' brains were fixed with 4 % paraformaldehyde for 48h, and a coronal section of 200 µm thickness was prepared to compare with matching slices in the rat's brain atlas of Paxinos and Watson [30] (Fig. 1d).

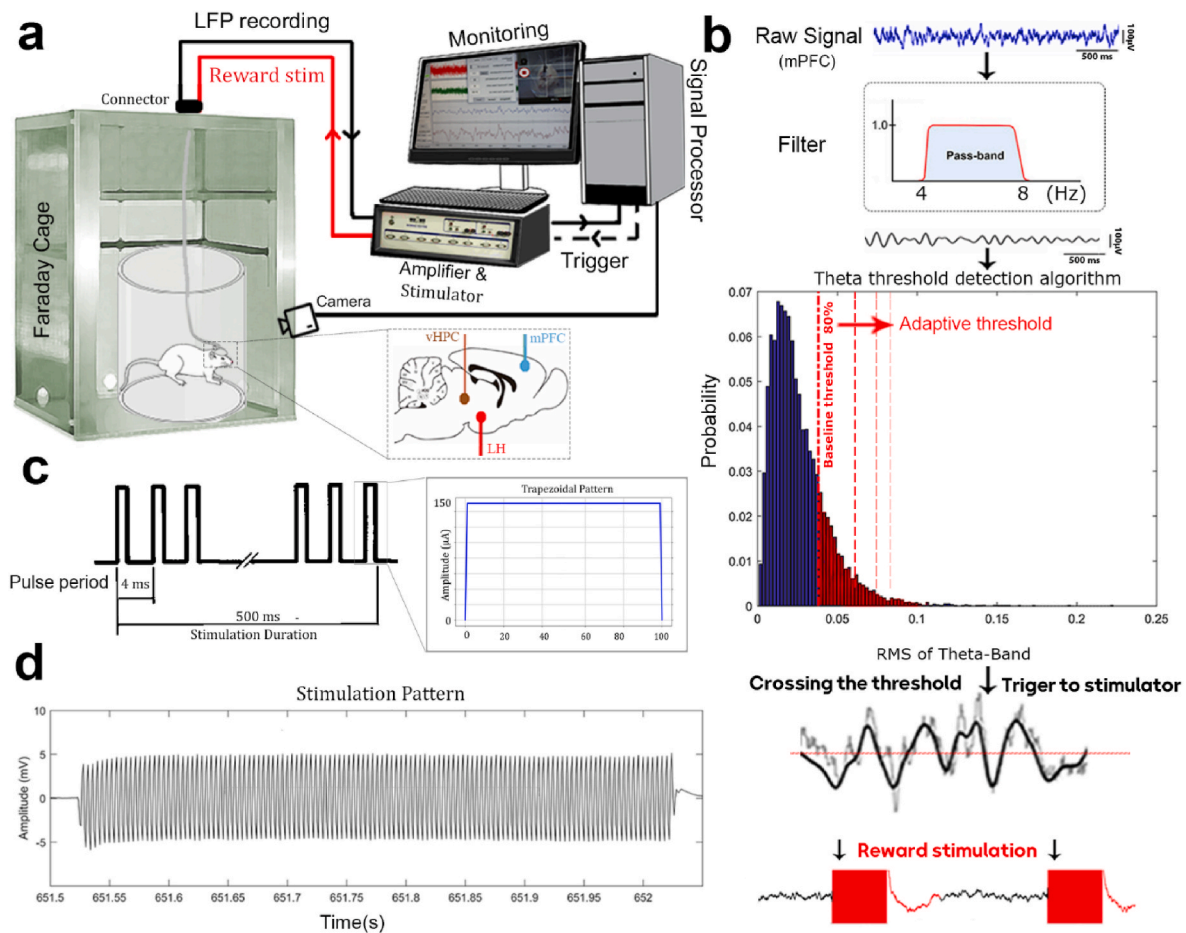
### 2.4. Feedback sessions

#### 2.4.1. LFP recording configuration

After one week of recovery from surgery, the previously implanted head socket was connected to the miniature buffer head-stage with high-input impedance for recording LFP signals (BIODAC-A, TRITA Health Tec., Tehran, Iran). The signals were amplified (1000 amplification gain), digitized at 1 kHz, and low-pass filtered <250 Hz via AC coupled with the recording system (BIODAC-ESR18622, TRITA Health Technology Co., Tehran, Iran). LFPs from mPFC and vHPC were recorded by using a fixed socket on the animal's head. The animals were permitted unrestricted movement during the feedback sessions. The rats in the OFF



**Fig. 1. Study design.** a) Timeline of the feedback sessions. b) Timeline of the behavioral sessions (with simultaneous LFP recording). c) Recording and stimulation design in each feedback session for the three groups. Contingent reward stimulation was carried out for the CL group. The obtained pattern of stimulation in each session of the CL group for each rat was delivered to the corresponding rat in the RL group (Dashed line). The OFF group did not receive any form of stimulation. d) Electrode implementation sites on rat skull and histological confirmation of sites in mPFC, LH, and vHPC. (LFP: local field potential; CL: closed-loop; RL: random-loop; mPFC: medial prefrontal cortex; LH: lateral hypothalamus; vHPC: ventral hippocampus).



**Fig. 2. Experimental set-up for adaptive closed-loop neurofeedback system.** a) Schematic representation of the CLNF. LFP recording of theta band RMS was obtained from mPFC, and reward stimulation was applied to LH. b) Distribution of theta band RMS showing the calculated threshold. Vertical dashed lines indicate the adaptive approach in applying the thresholds. The bottom panel displays a sample of theta activity evoked immediately after a single reward stimulation and prior to a following one. c) Sequence and wave patterns with an intensity of 150  $\mu$ A, 125 cathodal trapezoidal pulses with 100 $\mu$ s duration (1  $\mu$ s raising and falling times). The stimulation sequence in a duration of 500 ms. d) Recorded LFPs from mPFC during reward stimulation. (CLNF: closed-loop neurofeedback; LFP: local field potential; RMS: root mean square; CL: closed-loop; RL: random-loop; mPFC: medial prefrontal cortex; LH: lateral hypothalamus; vHPC: ventral hippocampus).

group did not receive any stimulation in these sessions. The procedure for CL and RL groups is described in detail respectively.

#### 2.4.2. Adaptive real-time feedback delivery of the LH stimulation in the CL group

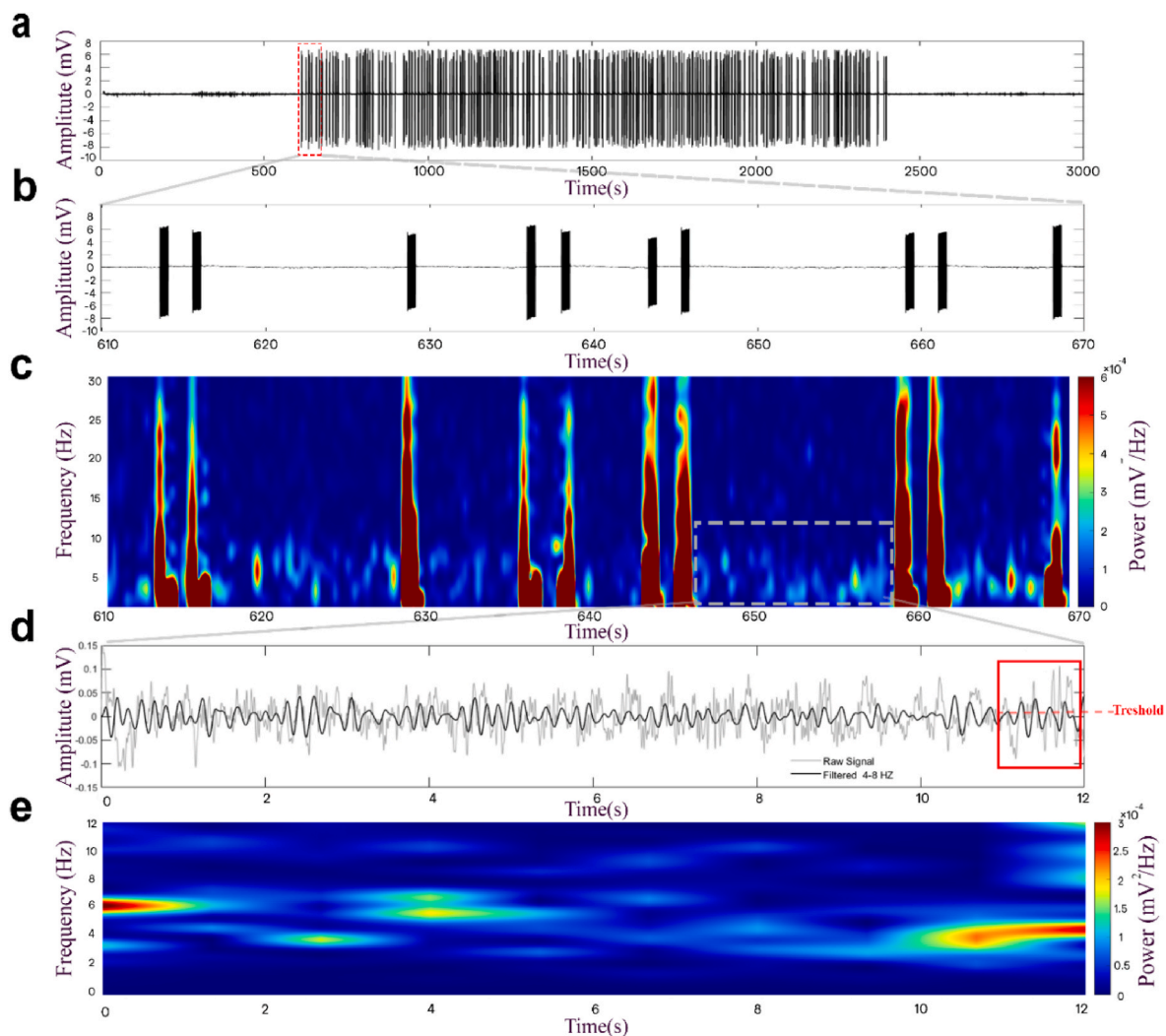
The training sessions were conducted in a cylindrical arena with a radius of 15 cm. We used a closed-loop system in which the spontaneous LFP activity of mPFC neurons was analyzed online at a sampling rate of 1 kHz and applied feedback reward stimulation to the LH (Fig. 2a). A single daily session of reinforcement theta training consisted of three consecutive blocks (10 min of pre-training; 30 min of up-regulation training; and 10 min of post-training) [28]. First, we collected LFP for 10 min and obtained the “prior” distribution of the area above the curves of individual theta root-mean-square (RMS). We used digital bandpass filtering (third-order Butterworth) and the value of the filtered signal to compute the band amplitude of 4–8 Hz oscillations online for every 50 ms bin. In the CL group, the RMS of the theta band was computed in the pre-training block, and the RMS value distribution was obtained using a custom-built MATLAB routine (Fig. 2b). The theta amplitudes proportional to 80 % of the RMS distribution were defined as the baseline threshold level for the training block. During the training block, theta band RMS was computed for every consecutive 500 ms (Fig. 2b). To

deliver adaptive feedback, when the calculated averages of RMS were larger than the baseline threshold (top 20 % of the prior distribution of RMS), this new average value (computed over the last 500 ms) was considered as the new threshold level. Subsequently, a rewarding electrical stimulus (frequency = 250 Hz, intensity = 150  $\mu$ A) [19,28] was delivered to the LH (Fig. 2c) with a 50 ms delay and lasting for 500 ms.

To prevent convolutions induced by reward stimulations, animals were not allowed to receive another reward within 500 ms following the previous stimulation. Furthermore, to avoid stimulation artifacts, the mentioned 500 ms duration period was excluded from the calculation of RMS distribution and threshold. Fig. 3 provides details of the stimulation and its impact on the mPFC signal.

#### 2.4.3. Non-brain state dependent feedback delivery of the LH stimulation in the RL group

The obtained pattern of stimulation in each session of the CL group for each rat was delivered to the corresponding rat in the RL group (non-contingent reward stimulation). Hence, both groups shared the same stimulation pattern, while the CL group received contingent rewards to theta band RMS level.



**Fig. 3.** The overall pattern of stimulations and its influence on raw signal. a) a representative excerpt of the raw signal is presented for a 50-min session, encompassing pre-training, training, and post-training stages. b) a 1-min segment during the training step, highlighting ten distinct stimulations. c) The corresponding time-frequency representation of panel (b). d) A segment of the raw signal is showcased during the inter-stimulation period. e) The associated time-frequency representation for the inter-stimulation segment in panel (c).

## 2.5. Behavioral sessions

### 2.5.1. Open field test

Two series of the open field tests were also conducted to measure locomotor and anxiety-like behavior in the groups that received stimulation [31]. Since the 'OFF' group did not receive stimulation, their locomotor activity was assumed to be equivalent to the pre-stimulation baseline of the other groups, and hence, was not separately recorded. The test area consisted of a  $60 \times 60$  cm square arena with 35 cm high walls. In the open field test, the rats were placed in the cube's center, then the movement traces were calculated for 10 min. We also calculated the thigmotaxic index as a measure of anxiety-related behavior [32, 33]. This index is determined by recording the time spent by rats in the peripheral zone of the arena divided by the total time spent in both the central and peripheral zones.

### 2.5.2. SWM task

Following the feedback sessions, animals were brought to the experimental room, where they stayed until the end of the experiments. During the behavioral assessment, the rats underwent food deprivation, reaching 85 % of their weight prior to behavioral training. At the beginning of each trial, food rewards were placed at the end of the two arms. If the rats re-entered the previously visited arm (during the sample run), they would not find any additional food, marking an incorrect trial. In contrast, during correct trials, the animals would find food at the end of the newly visited arm. For the DNMS task, we used a T-shape maze constructed with black Plexiglas (length: 50 cm, width: 10 cm, height: 25 cm). All animals were habituated to the T-maze for 10 min two days before the test session. Additionally, two shaping sessions (10 min) for the test procedures took place for two consecutive days. A ceiling-mounted video camera synchronized with LFP recording was used to capture all movements in the behavioral box.

Ten trials of the DNMS task were carried out daily for 10 consecutive days for all animals. Each trial of the DNMS consisted of two runs of "sample" and "choice." In the sample run, rats reached the center arm of the T-maze and were directed to one of the goal arms. A barrier was used for closing the other arm. After a short delay (~15 s), the barrier was opened, and the rats had to choose between two open-goal arms ("choice run") [14]. The correct trials were defined as those trials in which the rats reached the goal arm that was not previously visited. Two behavioral measures were used for the evaluation of the task performance. The percent of Correct Responses was defined as the number of (correct trials/total number of trials) \* 100. The time required to reach the goal arm's end was considered the latency.

## 2.6. Data analysis

### 2.6.1. Electrophysiology data analysis in the feedback sessions

A customized program developed by MATLAB version 2016b (MathWorks, Natick, MA, USA) was used to analyze the recorded LFPs during feedback sessions. LFP power spectral densities (PSDs) for each block were assessed. We implemented a strategy to eliminate the impact of electrical stimulation artifacts on analyzed data. Specifically, our analysis (PSDs, coherence, and signal correlation) was restricted to inter-stimulation periods, with an additional exclusion window of 500 ms following each stimulation event. This approach ensured that any potential confounding effects from electrical stimulation were removed, providing neural measures free from artifactual contamination. The Hanning window was multiplied by the raw LFP for a given block, and the PSD was computed using the p-Welch function. We applied the Hanning window to each window of data, not on the entire recording prior to applying Welch's method. The specific parameters used were a window size of 1 s and a window overlap of 250 ms. For coherence estimation we used the same window size and overlap as in the PSD estimation process. The frequency resolution of the resulting PSD was 0.05 Hz. PSDs for the pre-training (10 min) block and the post-training

(10 min) block were assessed. In a training block, the average of the theta band PSDs was extracted for the time windows between two consecutive reward stimulations. This PSD average was considered an index of the signal changes induced by contingent stimulations.

To analyze the signals, we first applied a bandpass filter to isolate theta range oscillations (4–8 Hz) for the purpose of computing coherence and correlation. In order to calculate the mPFC-vHPC coherence, magnitude-squared coherence was computed by using the "mscohere" function of MATLAB. The mean coherence values in the 4–8 Hz range were computed for each time interval (4s for pre- and post-training and inter-stimulation intervals for the training phase for bins of >500 ms). To evaluate the synchrony of the mPFC and vHPC theta oscillations, we first filtered the data in the theta frequency range, and then cross-correlation was analyzed by the "xcorr" function (with the "Coeff" option to normalize values) for the theta. The maximum absolute values were calculated for each time bin (again, 4s interval pre- and post-training and varied inter-stimulation intervals for the training phase for bins of >500 ms). The mean of these maximum values was considered as an mPFC-vHPC correlation. Fig. 4 provides an illustration of one feedback session recording and its results.

### 2.6.2. Electrophysiology data analysis in the last behavioral session

We used a specific epoch of the signals recorded during DNMS performance in the T-maze. Each epoch was selected 3s before and 1s after animals made decisions for the goal arm (Decision Point), both for correct and incorrect trials. Mean PSDs, coherence, and correlation of the mPFC-vHPC regions for sample and choice runs of each trial were obtained during these 4 s durations.

### 2.6.3. Statistical analysis

Two-way repeated measures ANOVA was used to compare the differences between groups over time. Post-hoc Bonferroni tests were used as appropriate (in some cases without considering significant interaction) to evaluate when performance changes became evident compared to the first session and how performance evolved over time. Our focus in such cases was on pinpointing the onset of sustained significant effects across the sessions. All data were presented as mean  $\pm$  SEM of the mean, and p-values less than 0.05 were considered statistically significant. GraphPad Prism Ver. 9.4.1 (GraphPad Software, Boston, Massachusetts USA, [www.graphpad.com](http://www.graphpad.com)) was used for the statistical analysis.

## 3. Results

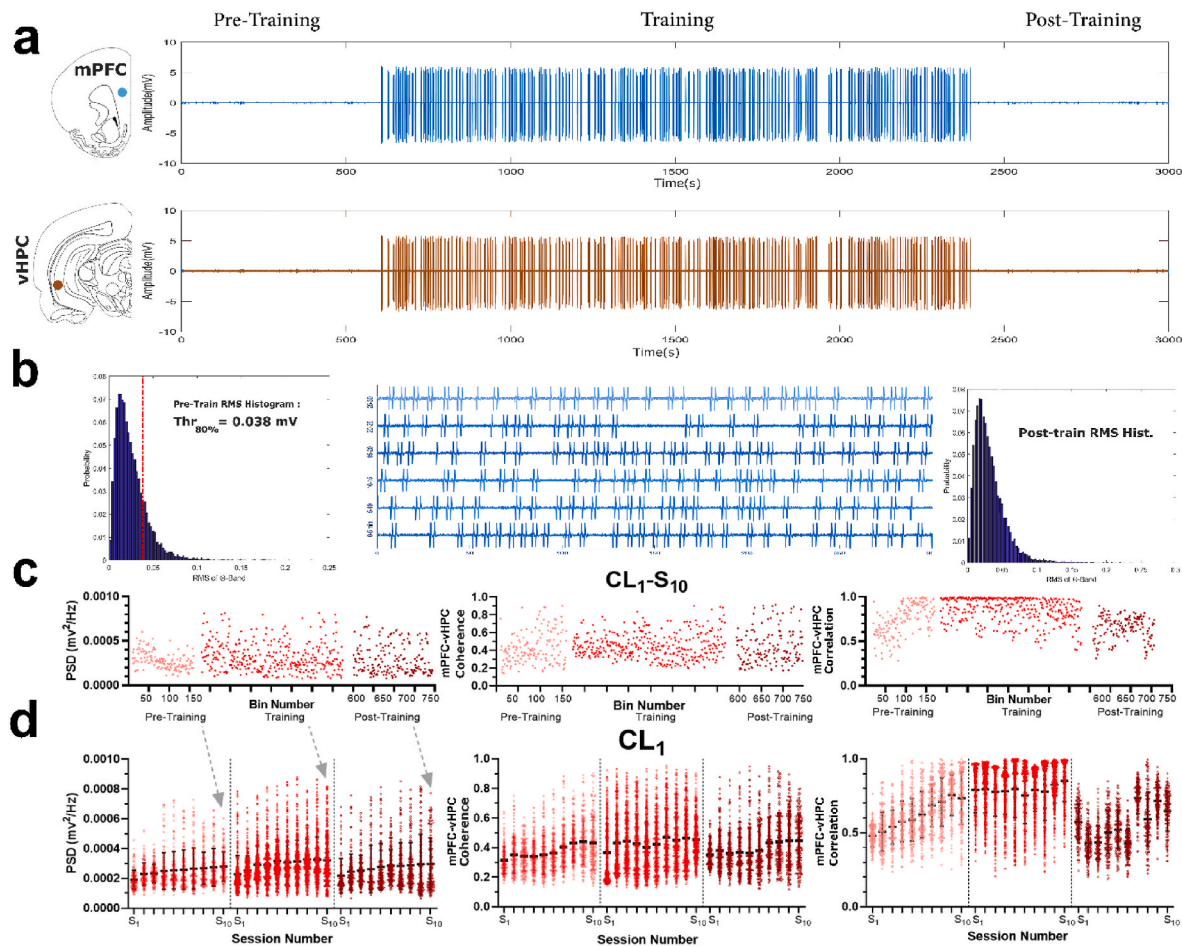
### 3.1. Feedback sessions

#### 3.1.1. Theta-CLNF increases the power of theta rhythm in mPFC

One-way ANOVA was conducted to compare mean PSDs in the theta (4–8 Hz) band before training across the three groups (OFF, CL, and RL), the results indicated no significant differences between the groups ( $F(2, 18) = 0.445, P = 0.645$ ). The main effect of the group was not statistically significant, suggesting that baseline theta power levels were similar across all groups.

Bonferroni's multiple comparisons test revealed no significant differences between any pairs of groups. Specifically, the comparison between the OFF and CL groups was not significant ( $t = 0.42, P > 0.99$ ). Similarly, the OFF versus RL comparison showed no significant difference ( $t = 0.53, P > 0.99$ ). The comparison between CL and RL also did not reach significance ( $t = 0.94, P > 0.99$ ). These results indicate that there were no significant baseline differences in theta power among the groups before training.

Then, we compared the three groups in terms of mean PSDs in the theta band over all sessions using a two-way repeated measure ANOVA. The session steps (pre-training, training, and post-training) were considered as the within-group factor. The interaction effect of group  $\times$  training step was found to be significant ( $F(4, 36) = 8.76, P < 0.0001$ ). Our results showed a significant difference between the groups in terms



**Fig. 4.** A sample of one feedback session recording and results. a) LFP recording samples in pre-training, training, and post-training for mPFC and vHPC. b) Theta-RMS distribution in the pre-training phase (left), distribution of reward stimulation in 5 min bins in the training phase (middle), and theta-RMS distribution in the post-training phase (right). c) PSD of the mPFC (left), theta-range mPFC-vHPC coherence (middle), and correlation (right) distribution samples (one subject in the CL group) in one feedback session for the three steps (Pre-training, training and post-training) and d) for all sessions. (LFP: local field potential; RMS: root mean square; CL: closed-loop; mPFC: medial prefrontal cortex; vHPC: ventral hippocampus; PSD: power spectrum density).

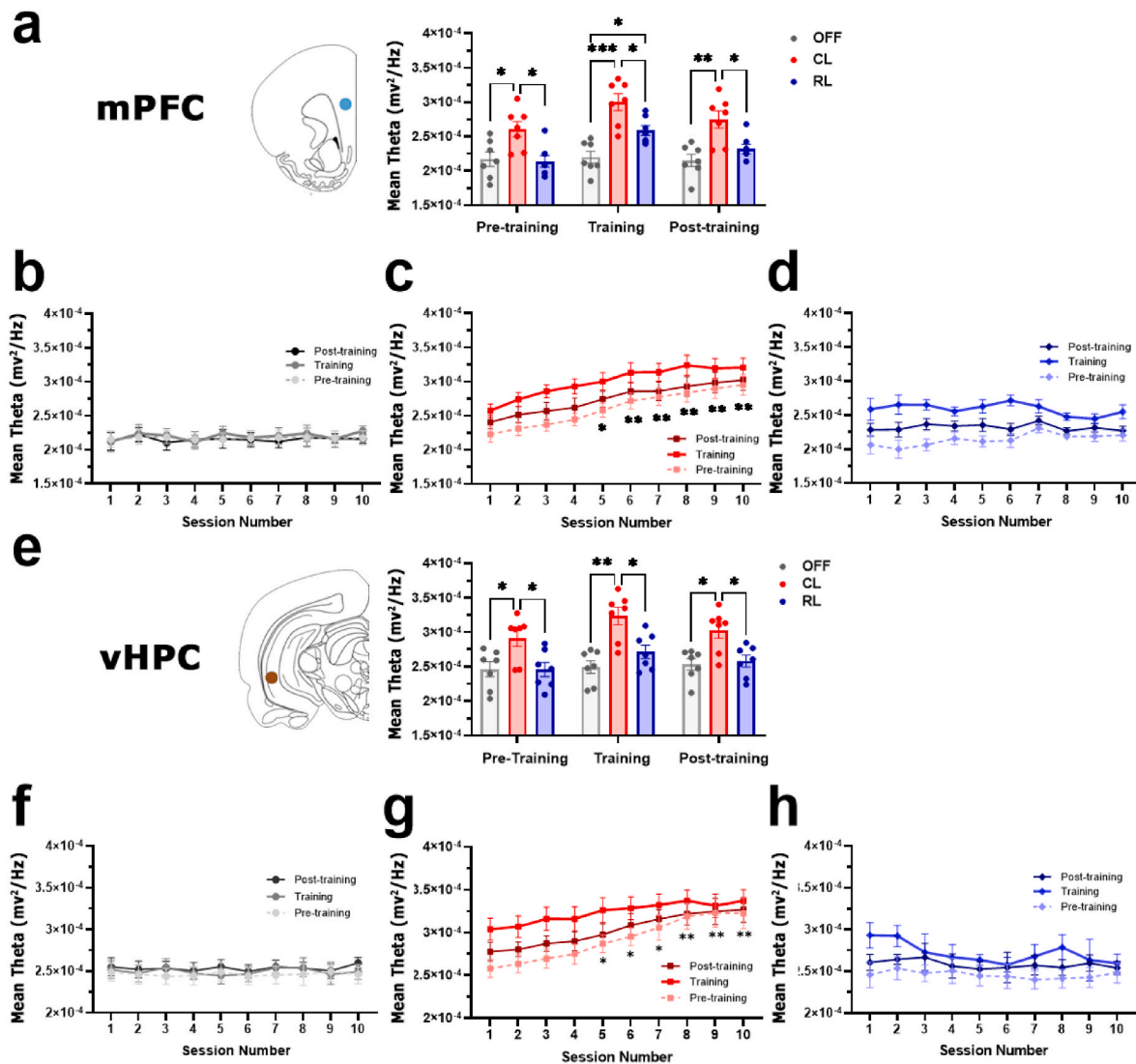
of mPFC theta power ( $F(2, 18) = 11.53, P < 0.001$ ). The main effect of the training step was found to be significant ( $F(2, 36) = 43.56, P < 0.0001$ ). *Post-hoc* Bonferroni tests revealed a significant difference between the CL group and the other two groups in three steps of all the sessions' means (see Supplementary Materials; Table S1). There was also a significant difference between the OFF and the RL groups in the training step (Fig. 5a). These findings support our hypothesis that enhancing the power of theta oscillations contingent on mPFC theta through a CLNF leads to an increase in theta rhythm power in this region.

Subsequently, we analyzed the theta power changes using mean PSDs for each group separately in mPFC and compared the theta power changes in consecutive sessions in three steps of LFP recording (pre-training, training, post-training) using repeated-measures ANOVA (see Fig. 5). As reflected in Fig. 5c a significant increase was observed in the mean theta power of the CL group during the course of feedback sessions ( $F(2.04, 12.23) = 46.95, P < 0.0001$ ). Bonferroni multiple comparisons was used to evaluate the related sustained changes of theta power (see Fig. 5c; details in Supplementary Materials). The findings revealed a significant increase in the theta power during pre-training (since session 5th), training (since 2nd session) and post-training (since session 6th) steps of the sessions compared to the first session in the CL group. Similar analysis comparing the theta power changes revealed non-significant effects for the OFF ( $F(2.81, 16.84) = 0.52, P = 0.66$ ) and RL ( $F(3.01, 18.05) = 1.24, P = 0.32$ ) groups (Fig. 5, b: OFF; d: RL).

### 3.1.2. Theta-CLNF of mPFC increases the power of theta rhythm in vHPC

We compared the three groups in terms of mean PSDs over all sessions using a two-way repeated measure ANOVA. The session steps (pre-training, training, and post-training) were considered as the within-group factor. The interaction effect of group x training step was found to be significant ( $F(4, 36) = 2.76, P < 0.05$ ). The main effect of the training step was found to be significant ( $F(2, 36) = 13.00, P < 0.0001$ ). Our results showed a significant difference between the groups in terms of theta power ( $F(2, 18) = 9.65, P = 0.0014$ ). *Post-hoc* Bonferroni tests revealed a significant difference between the CL and the other two groups in all three session steps (see Supplementary Materials; Table S3). However, no significant difference existed between the OFF and the RL groups in any of the three steps (Fig. 5e). These findings support our hypothesis that enhancing the power of theta oscillations contingent on mPFC theta through a CLNF also leads to an increase in theta rhythm power in vHPC.

Subsequently, we analyzed the theta power changes using mean PSDs for each group separately in vHPC and compared the theta power changes in consecutive sessions in three steps of LFP recording (pre-training, training, post-training) using repeated-measures ANOVA (see Fig. 5). As reflected in Fig. 5g a significant increase was observed in the mean theta power of the CL group over sessions ( $F(1.91, 11.44) = 57.95, P < 0.0001$ ). Bonferroni multiple comparisons was used to evaluate the related sustained changes of theta power. The findings revealed a significant increase in the theta power during pre-training



**Fig. 5.** Mean PSDs as indices of signal changes in Pre-training, Training, and Post-training sessions for OFF (gray), CL (red), and RL (blue) groups obtained from mPFC and vHPC. a) Groups' comparisons between the mean PSDs in mPFC for all sessions. b, c and d) Mean mPFC PSDs for the OFF, CL and RL groups, respectively. e) Groups' comparisons between the mean PSDs in vHPC for all sessions. f, g and h) Mean vHPC PSDs for the OFF, CL and RL groups, respectively. (CL: closed-loop; mPFC: medial prefrontal cortex; vHPC: ventral hippocampus; PSD: power spectrum density). (\*:  $p < 0.05$ ; Mean  $\pm$  SEM).

step of the sessions compared to the first session in the CL group (see details in Supplementary Materials; Table S4). Similar analysis comparing the theta power changes revealed non-significant effects for the OFF ( $F(3.92, 23.54) = 0.094, P > 0.05$ ) and RL ( $F(3.94, 23.64) = 1.09, P > 0.05$ ) groups (Fig. 5, f: OFF; h: RL).

These results demonstrate that our theta-CLNF had a significant effect on the power of theta in both mPFC and vHPC regions after the training sessions. Findings demonstrate that as theta upregulation training progresses, there is a gradual increase in the power of the theta. Moreover, these changes in theta power were sustained after the completion of the training sessions in the CL group.

Fig. 6 shows the PSDs for the three groups (CL, OFF and RL) in the mPFC across the frequency spectrum up to 50 Hz. The PSDs are presented for both pre-training and post-training steps state.

### 3.1.3. Theta-CLNF of mPFC leads to an increase of the functional connectivity in the mPFC-vHPC circuit in the range of theta rhythm

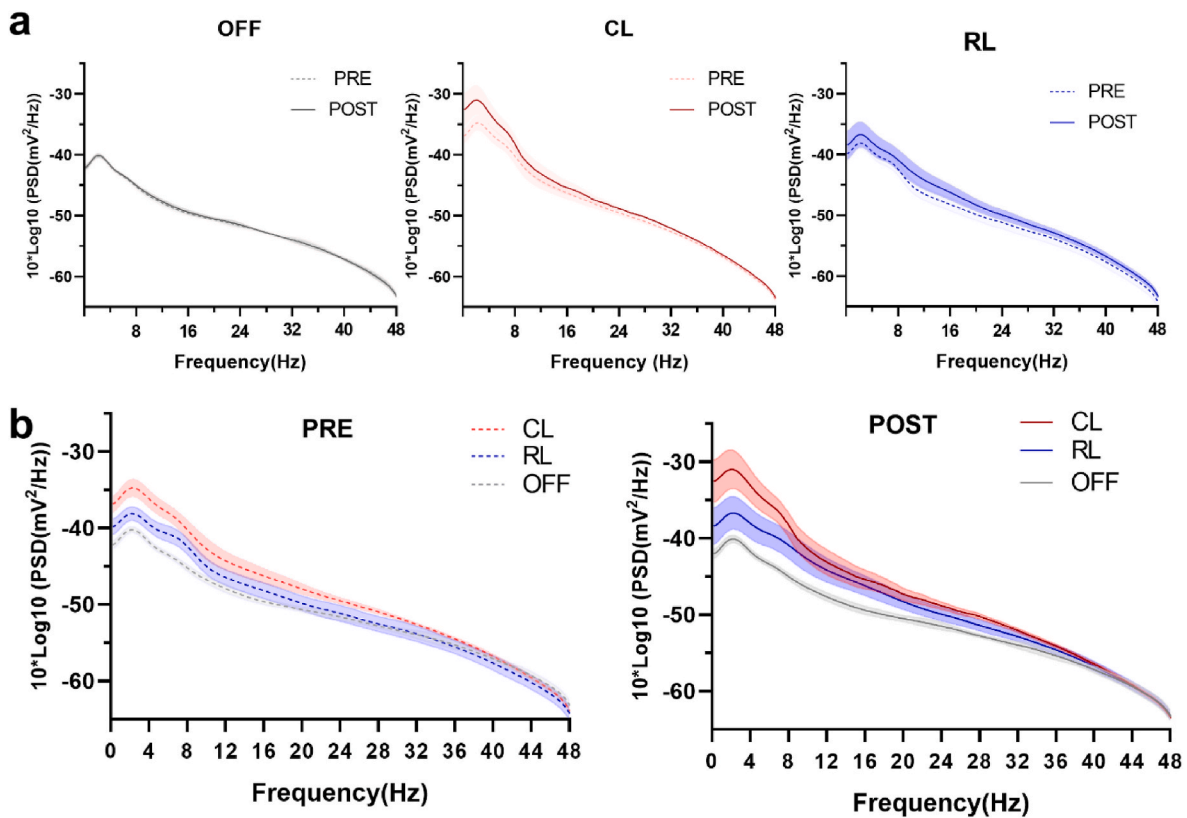
**3.1.3.1. mPFC-vHPC coherence.** We compared the mean coherence data of all three groups using two-way ANOVA repeated measurements. The results indicated a significant difference between the groups ( $F(2,18) =$

$27.97, P < 0.001$ ) (Fig. 7b). Bonferroni multiple comparisons indicated a significant difference between the CL and the other two groups in all three steps. Additionally, there was a significant difference between the OFF and the RL groups in the training step (Fig. 7c).

Mean coherence for all groups over sessions in three steps of LFP recording (pre-training, training, post-training) was computed. As reflected in Fig. 7f, a significant increase was observed in the mean coherence of the CL group over sessions ( $F(2.56, 15.34) = 31.20, P < 0.0001$ ). Bonferroni multiple comparisons revealed a significant increase in pre-training mean coherence compared to the first session in the CL group. Similar analysis comparing the theta coherences revealed non-significant effects for the OFF ( $F(2.32, 13.93) = 1.07, P > 0.05$ ) and RL ( $F(3.12, 18.75) = 3.04, P > 0.05$ ) groups (Fig. 7e: OFF; 7g: RL). (see details in Supplementary Materials; Table S5).

These findings show that strengthening the power of theta rhythm through the CLNF system in mPFC increases the coherence in the range of theta rhythm between the mPFC and vHPC regions.

To control for the potential influence of oscillatory power differences on coherence, the regression analysis revealed significant group differences in coherence and a significant interaction between group and oscillatory power, but no confounding effect of oscillatory power was



**Fig. 6.** PSDs for Pre and Post Training over all Feedback Sessions. a) PSD plots for each group (CL, OFF and RL) showing pre-training and post-training power spectra in the mPFC. The PSDs are plotted across the frequency spectrum up to 50 Hz. b) Comparative PSD plots for the three groups at pre-training and post-training. This panel illustrates the overall effect of stimulation on the spectral power distribution. (CL: closed-loop; RL: random-loop; mPFC: medial prefrontal cortex; PSD: power spectrum density).

observed (see Supplementary Materials, Table S8). Fig. 8 shows the coherence spectra for the all groups across the frequency spectrum up to 50 Hz. The coherence spectra are presented for both pre-training and post-training states.

**3.1.3.2. mPFC-vHPC correlation.** We compared the mean correlation data of all three groups using two-way ANOVA repeated measurements. The results indicated a significant difference between the groups ( $F(2,18) = 22.71, P < 0.0001$ ). Bonferroni multiple comparisons indicated a significant difference between the CL and the other two groups in all three steps. Additionally, there was a significant difference between the OFF and the RL groups in the training step (Fig. 7c).

The mean correlation for all groups over sessions in three steps of LFP recording (pre-training, training, post-training) was computed. As reflected in Fig. 7i, a significant increase was observed in the mean correlation of the CL group over sessions ( $F(2,28, 13.70) = 15.43, P < 0.001$ ). Bonferroni multiple comparisons revealed a significant increase in pre-training mean correlation compared to the first session in the CL group. Similar analysis comparing the theta coherences revealed non-significant effects for the OFF ( $F(2,32, 13.93) = 1.07, P > 0.05$ ) and RL ( $F(3,12, 18.75) = 3.04, P > 0.05$ ) groups (Fig. 7h; OFF; 7j; RL). (see details in Supplementary Materials; Table S7).

These findings reveal that strengthening the power of theta rhythm through the closed-loop neurofeedback system in mPFC increases the correlation in the range of theta rhythm between the mPFC and vHPC regions.

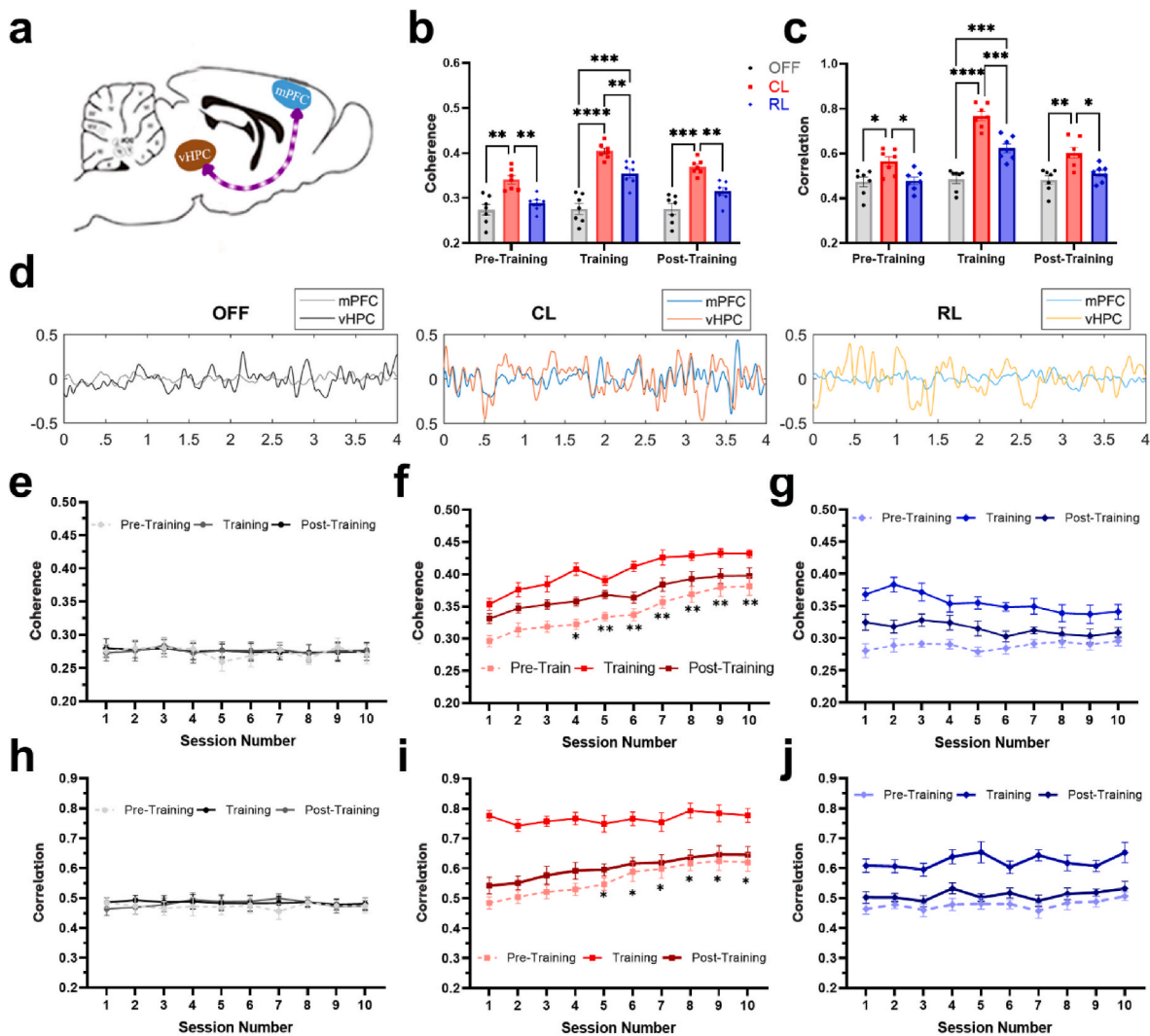
### 3.2. Behavioral sessions

Finally, we examined how modulating theta activity affects behavioral performance in an SWM task by comparing correct and incorrect

responses in sample and choice runs. Furthermore, response latencies were analyzed as another important measure of performance in SWM. Note that no stimulation was delivered during the behavioral sessions.

#### 3.2.1. Theta-CLNF leads to an increase of the correct responses in the SWM

The percentage of correct responses in the choice run was determined for each session in the three groups based on the T-maze task performance. The results of the repeated measure ANOVA indicated a significant group effect ( $F(2, 18) = 27.55, p < 0.0001$ ) and session effect ( $F(3.61,65) = 12.52, p < 0.0001$ ), but no significant group  $\times$  session interaction effect was observed ( $F(18, 162) = 0.16, p > 0.05$ ). It is reported that post-hoc analyses on non-significant interactions might be informative when the main effects of interest in an interaction are significant [34,35]. Given that the effect of session was critical to our research question, despite the non-significant interactions involving groups, separate paired t-tests were conducted between groups to explore the learning effect over sessions. Although the CL group showed an increase in the percentage of correct responses in all sessions compared to the OFF group, the difference was greater in the last sessions. Fig. 9 shows that the RL group performed worse in all sessions than the OFF group. Bonferroni's multiple comparisons test revealed significant differences between CL and other two groups in last sessions (RL:  $t(12) = 6.00, p < 0.001$ , OFF:  $t(12) = 2.61, p < 0.05$ ) (Fig. 9b-left). Furthermore, comparing the performance of rats in the three groups over ten sessions showed a significant difference in the mean rate of correct responses ( $F(1.28, 7.67) = 25.4, p < 0.001$ ) (Fig. 9b-right). Bonferroni's multiple comparisons test revealed significant differences between CL and OFF ( $t(6) = 4.94, p < 0.01$ ); CL and RL ( $t(6) = 5.67, p < 0.01$ ) and, OFF and RL ( $t(6) = 4.10, p < 0.05$ ).



**Fig. 7.** Functional connectivity of the mPFC-vHPC circuit during the time course of feedback sessions. a) Schematic representation of mPFC-vHPC functional connectivity. b) Theta-range mean coherence of all sessions for three groups in the three steps. c) Theta-range mean correlations of all sessions for three groups in the three steps. d) LFP samples during the training phase. e, f and g) Coherence changes in the OFF, CL and RL groups, respectively. h, i and j) Correlation changes in the OFF, CL and RL groups, respectively. (Panels f and i show significant increase of the mean coherence compared to the first session in pre-training for the CL group) (CL: closed-loop; mPFC: medial prefrontal cortex; vHPC: ventral hippocampus; PSD: power spectrum density).

### 3.2.2. Theta-CLNF leads to an increase in the latencies in the SWM

To evaluate the data related to the time to reach the target arm, variance was analyzed using the intersection of the grouping variable (three groups) and the within-group variable (10 sessions). Results exhibited a statistically significant difference in the time to reach the target arm between the three groups ( $F(2, 18) = 37.47, P < 0.0001$ ). Additionally, there was a significant difference in the time to reach the target arm during the ten sessions ( $F(2.49, 44.47) = 15.04, P < 0.0001$ ). The lack of significance in the interaction between variables suggested a similar trend across all three groups ( $F(18, 162) = 0.51, P > 0.05$ ). Further, comparing the performance of rats in the three groups over ten sessions showed a significant difference in the mean latencies ( $F(1.31, 7.86) = 39.77, p < 0.001$ ) (Fig. 9c-right). Bonferroni's multiple comparisons test revealed significant differences between CL and OFF ( $t(6) = 4.16, p < 0.05$ ); CL and RL ( $t(6) = 3.53, p < 0.05$ ) and, OFF and RL ( $t(6) = 14.95, p < 0.0001$ ). Overall, these findings show that the OFF group had the shortest time to reach the target arm, while the RL group had the longest time during all sessions (Fig. 9c-right). A two-way repeated measures ANOVA was conducted to investigate the effects of trial type and group on performance in correct and incorrect trials. The analysis revealed a significant main effect of group,  $F(1.631, 19.57) =$

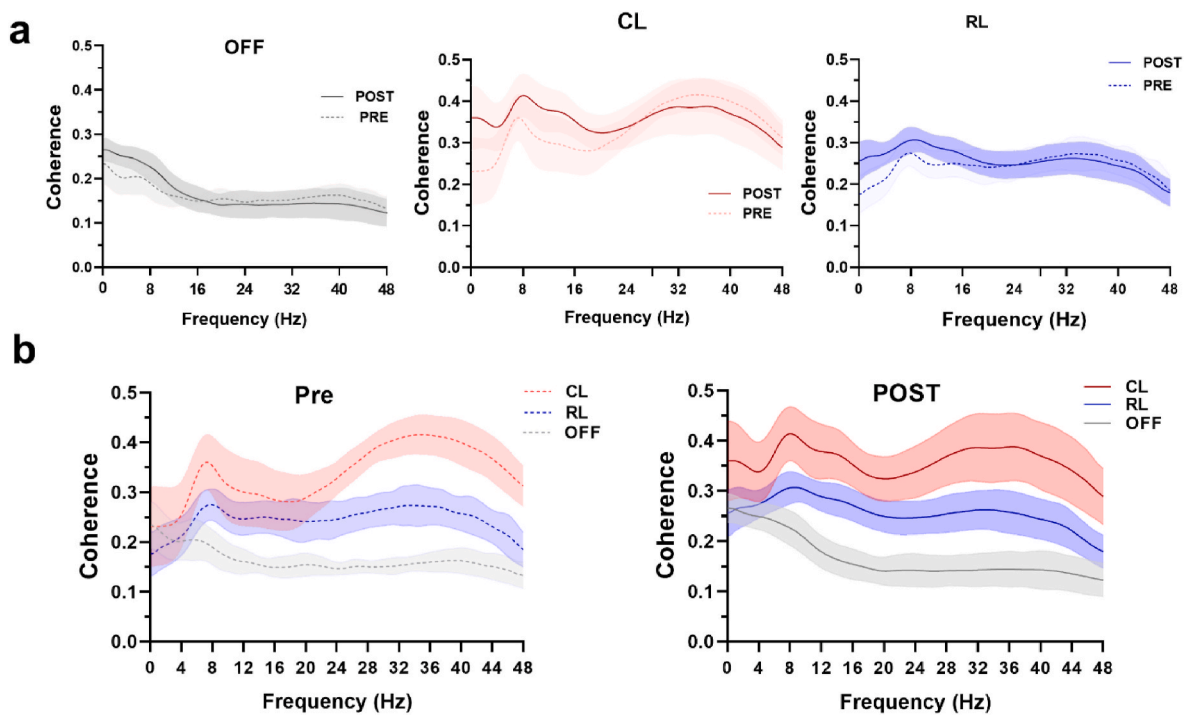
44.47,  $p < 0.0001$ , indicating substantial differences between groups. However, no significant effects were found for trial type,  $F(1, 12) = 1.742, p = 0.2115$ , or the interaction between trial type and group,  $F(2, 24) = 0.7023, p = 0.5054$ .

Post-hoc Bonferroni tests showed that, in correct trials, the OFF group performed significantly worse than both the CL ( $p = 0.0179$ ) and RL ( $p < 0.0001$ ) groups, and the CL group performed significantly worse than the RL group ( $p = 0.0374$ ). In incorrect trials, the OFF group also performed significantly worse than both the CL ( $p = 0.0153$ ) and RL ( $p = 0.0010$ ) groups, but there was no significant difference between the CL and RL groups ( $p = 0.9877$ ). These findings indicate that group membership had a significant impact on performance, while trial type did not, and the differences were consistent across trial types.

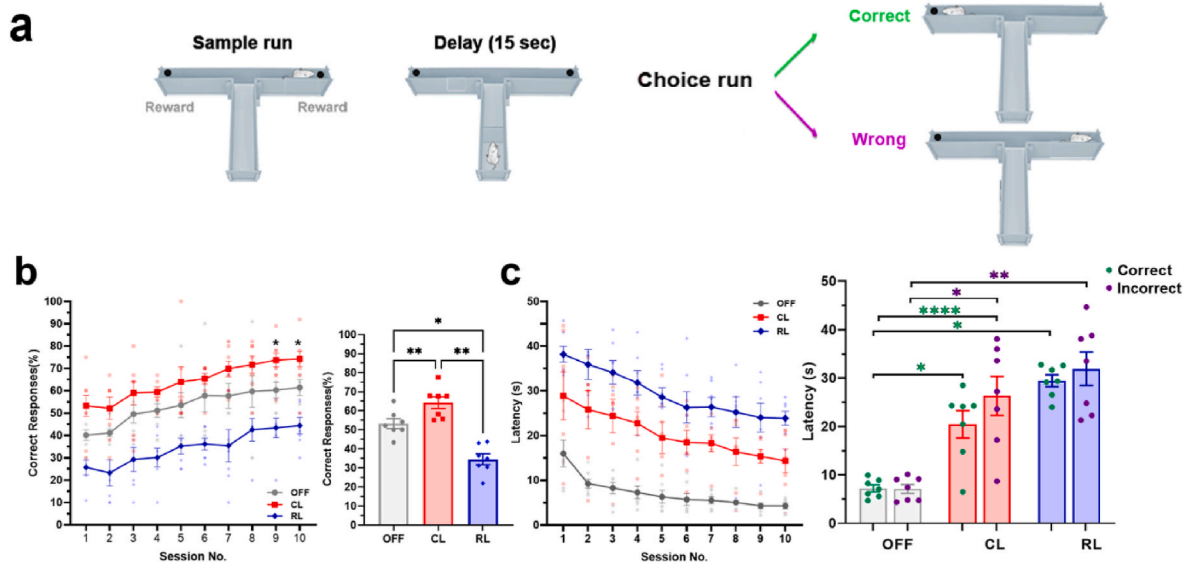
Intriguingly, our results unmasked a significant correlation between pre-to-post training changes in theta and performance in the SWM task.

### 3.3. Electrophysiological evidence for behavioral changes induced by the CLNF

A series of statistical analyses were conducted on the LFP recording of the 10th behavioral session to compare the effects of different



**Fig. 8.** Coherence Spectra for Pre and Post Training over all Feedback Sessions. a) mPFC-vHPC coherence spectra for each group (CL, OFF, and RL) illustrating pre-training and post-training. b) Comparative coherence spectra for the three groups at pre-training and post-training. (CL: closed-loop; RL: random-loop; mPFC: medial prefrontal cortex).



**Fig. 9.** Performance in SWM for all behavioral sessions. a) T-maze used for evaluating SWM performance. b) Percentage of correct response over behavioral sessions for groups (left) and mean response rate over all sessions (right). c) Latency in choice run in each session for groups (left) and mean Latency for three groups regarding correct and incorrect responses (right). (SWM: Spatial Working Memory).

interventions in groups considering channels (mPFC, vHPC), trial types (correct, incorrect), and Task phases (sample, choice) on various measured factors, including z-score PSD, coherence, and correlation. The results are summarized in Fig. 10.

### 3.3.1. Theta power

#### 3.3.1.1. mPFC

3.3.1.1.1. *Sample run.* To evaluate the role of theta power of mPFC during the *sample run* among three groups, A mixed-effects model

analysis of variance (ANOVA) were conducted. Significant main effects were found for response type,  $F(1, 205) = 22.43, p < 0.0001$ , and group,  $F(2, 205) = 17.86, p < 0.0001$ , but not for their interaction,  $F(2, 205) = 0.1053, p = 0.9001$ . Post-hoc Bonferroni tests revealed significant differences for correct trials between OFF and CL ( $p < 0.0001$ ) and CL and RL ( $p < 0.01$ ), but not between OFF and RL. For incorrect trials, significant differences were found between OFF and CL ( $p < 0.01$ ) and CL and RL ( $p < 0.05$ ), but not between OFF and RL. These results indicate that the CL group differs significantly from the others, with higher theta power in both correct and incorrect trials, especially in incorrect trials