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Phase-amplitude modulation during critical period plasticity in mouse visual cortex*

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***Abstract*— Much of our understanding of experience-dependent plasticity originates from the level of single cells and synapses through the well-established techniques of whole-cell recording and calcium imaging. The study of cortical plasticity of neural oscillatory networks remains largely unexplored. Cross-frequency coupling has become an emerging tool to study the underlying mechanisms for synchronization and interaction between local and global processes of cortical networks. The phase of low-frequency oscillations modulates the amplitude of high-frequency oscillations through a phase-amplitude coupling. Recent studies found that gamma-band oscillations associate with critical period plasticity. The existence of such mechanisms in ocular dominance plasticity is yet to be fully demonstrated. In this study, in-vivo electrophysiological methods for recording local field potentials in the primary visual cortex (V1) of anesthetized mice are employed. Our results reveal the mechanisms of neuronal oscillatory activities for the experience-dependent plasticity of developing visual cortical circuits.**

Index terms- Local field potential, neural oscillation, ocular dominance plasticity, phase-amplitude coupling, primary visual cortex.

I. INTRODUCTION

Cross-frequency coupling (CFC) may serve a functional role in neuronal computation, learning, and communication. It is used to study the interaction of brain rhythms in different frequency bands [1]. This interaction is reflected in the synchronous neural oscillations and is known to be a fingerprint of functional coupling. Phase-amplitude coupling (PAC), a type of CFC whereby the amplitude of high-frequency oscillations (HFOs), such as gamma, is modulated by the phase of low-frequency oscillations (LFOs), such as delta and theta [2]. This communication between different frequencies integrates their local information flow into a common brain network. A large number of studies have been conducted using CFC to explicate functional activity of the brain underlying e.g. perception, learning, sensory and working memory [3]. Intriguingly, within the visual system, PAC coupling acts as a mechanism for the dynamic coordination of brain activity over multiple spatial scales, with high-frequency activity coupled to low-frequency phase synchrony [4, 5]. Electrophysiological recording from different spatial scales includes the measurement of synchronized population activity such as local field potential (LFP), reflecting the activity of several tens of thousands of nerve cells [6]. Information and processing is integrated across these spatial scales and a hierarchy of mutually interacting oscillations is synchronized [1, 7].Visual stimuli modulate the amplitude of the activity-induced ongoing oscillations in the V1 recorded using LFP signals [8]. It has been reported that gamma power is modulated with the theta phase to visual stimuli leading to strong attention effects in the visual cortex (V1) [9].

PAC is not only well studied in humans, monkeys but lower species such as rats and mice [10]. In rodents, during critical period (CP) plasticity changes in gamma rhythm are observed. The experience-dependent refinement of the cortical circuits is restricted to CP of plasticity. All the neuronal connections are malleable during this period. Towards the end of the CP, the experience-dependent plasticity of the neural circuitry ceases to occur [11]. It has been observed that eyelid closure during the CP drives a robust transient peak in gamma power [12]. Both the initiation of this oscillatory activity and experience-dependent plasticity are dependent on the level of inhibition in the circuit [13, 14]. The enhancement of PAC during critical period monocular deprivation (CPMD), is suggestive of the formation and strengthening of new synchronous neural connections in the non-deprived eye after MD [12]. Previous studies also reported that Ketamine-Xylazine (KX) anesthetized mice exhibited pronounced delta rhythms, markedly increased power at delta frequencies [15, 16]. The relationship between neural synchrony, cognition, and the perceptual process has been widely studied, however, less is explored in the possible role of neural synchrony in amblyopia.

In this study, we aim to provide evidence for important changes in parameters of neural synchrony during juvenile and adulthood after 4 days of MD. The visual stimuli presented to anesthetized mice activate the V1 neurons by the interaction within functionally coupled neuronal oscillations in a phase-amplitude manner (PAC).

II. MATERIAL AND METHOD

A. Animals

Adult male/female wild-type (WT) C57BL/6 mice (n=10) were used. In-house animal breeding was done in the Laboratory Animal Research Unit at the City University of Hong Kong. The mice were housed under a 12:12 light/dark cycle, kept in individually ventilated cages (IVC). In each cage, 5 mice were kept and allowed free access to food and water. All experimental protocols were approved by the Animal Research Ethics Sub-Committees of City University of Hong Kong and the Department of Health, HKSAR. For both the WT adult MD group and CPMD group, 5 animals

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with age P60 and P23 respectively, were used for the experimentation for each group.

B. Short term monocular deprivation & surgical procedures

For short term MD, one eye was closed at postnatal day 23 (P23) in juvenile and P60 in adulthood using a single suture tied with 6-0 nylon surgical suture needle with thread (Ningbo Medical Needle Co Ltd, China) under brief anesthesia KX combination (Ketamine: 100mg/Kg, Xylazine: 10 mg/Kg; Alfasan International B.V., Holland). The suture was removed 4 days later at P28 and P65 with fine iridectomy scissors and the eyes were flushed with sterile saline. During the surgical procedure mice's body temperature was maintained at 37°C by a homeostatically controlled heating pad. Following the induction of anesthesia (K-X combination), the mice were mounted on a stereotaxic instrument (RWD Life Science, Shenzhen, China). The stereotaxic instrument was adjusted to place the bregma and lambda on a flat skull surface (AP:-3.2 to -4.8 mm, ML: 3.0 to 3.8 mm, relative to bregma). A piece of the scalp over the primary V1 was removed to expose the cortex, and for reference electrode, a bone screw was fixed in the frontal bone. The exposed cortical surface was then covered with extracellular saline to prevent drying. The electrode was lowered into the brain to an appropriate depth (V1: <1mm) and recording was started after 30 min. In each mouse, three to four separate penetrations were spaced evenly at least 200 µm apart across the binocular region of V1. The eyes were kept moist with ophthalmic lubricant ointment until recording. Mice were euthanized at the end of recording by Dorminal (300mg/kg).

C. Visual stimuli

The visual stimulus was generated with a MATLAB (R2012b, Math Works, US) script based on a package called Psychtoolbox-3 (PTB-3) (Brainard, 1997) on an LCD monitor with LED backlight (P2311Hb, Dell, US). To obtain maximum response for those cells with orientation selectivity, bars of light with varying size and orientation were presented. In the case of spatial acuity, visual stimuli of sinusoidal drifting gratings of increasing orientation angles (0-330º) with 0.04 cycle/degree in random order, repeated 10 cycles. The movie duration was 1.5 s drifting sinusoidal grating and 1 s break at temporal frequency 2 Hz. Optimal stimuli were presented to either eye alternatively, and the relative strength of response was determined.

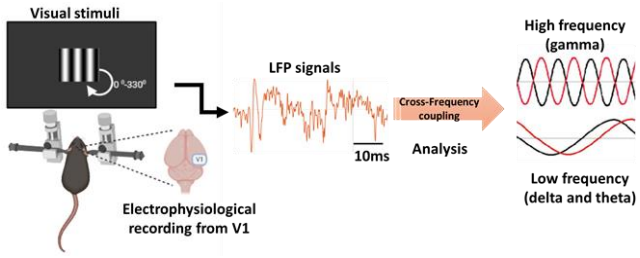

Fig.1. A schematic diagram of electrophysiological recording from the visual cortex. Polyimide coated Platinum/Iridium ribbon microelectrode array was used to record the LFPs signals from the V1.

D. Local Field Potential recording

Recordings were performed with Polyimide coated Platinum/Iridium (70:30) ribbon microelectrode array Clunbury Scientific LLC, USA) with tip resistances of 30–50 kΩ (Fig. 1). The A-M Systems 3600 (A-M Systems, US) amplifier and CED Micro 1401-3 (Cambridge Electronic Design, UK) data acquisition system were used. The signals were sampled at 25 kHz and band-pass-filtered 0.3-300 Hz for LFP, amplified and fed to spike2 software (Cambridge Electronic Design, UK) and Matlab for offline analysis. To remove the 50 Hz noise of the power line, a notch filter was applied off-line to LFP signals. We sampled neuronal signals from 16 recording sites in a single recording session.

E. Data Analysis

To assess the influences on neural oscillatory synchrony after 4 days MD during the CP in mice, CFC between LFOs and HFOs was analyzed by the PAC method and the modulation index (MI) was computed based on the Kullback-Leibler MI estimation [17]. Firstly, the raw data is filtered by using a linear finite impulse response (FIR) filter into a lower frequency band (1-29 Hz) for phase and a higher frequency band (10-300 Hz) for amplitude. For each filtered signal, the time series of the phases of a lower-frequency band and the amplitude envelope of a higher-frequency band of the LFP signal are extracted by implementing the standard Hilbert transform. Secondly, the phases were binned into eighteen intervals (from 0º to 360º with step 20º). The mean of the amplitude envelope over each phase bin was calculated to quantify the amplitude distribution over phase bins. Lastly, the MI was computed by normalizing H (the entropy measure of the normalized amplitude distribution over phase bins) by the maximum possible entropy value Hmax = log(N), where N equals to the no. of phase bins, as shown in the following equation:

$$\boldsymbol{MI} = \frac{\boldsymbol{H_{max} - H}}{\boldsymbol{H_{max}}} \qquad (1)$$

The average MI was calculated for all trials in each group. To test the statistical significance of the MI values, a distribution of 50 surrogate MI values was created by randomly shuffling the composite time series of high-frequency amplitude envelope and phases of a low-frequency signal after segmenting equally into 20 blocks. Assuming the surrogate MI values are normally distributed, the MI value of the original signal is considered significant if it reached the top 5% of this distribution of surrogate data, otherwise, it is ignored and replaced with zero.

F. Statistical Analysis

The number of electrode sites (LFP files) used for the group WT adult MD (P60-P65) and Juvenile CPMD (P23-P28) were 128 and 137 respectively. Mean of Modulation Index (MI)±SEM is calculated and is normalized by 10^{-3}. Statistical analysis was done using GraphPad prism software version 8.0.1. All *p*-values were determined with paired 't' test except otherwise stated.

III. RESULTS

A. Effect of monocular deprivation on phase-to-amplitude modulations during the critical period

The change in neural oscillatory synchrony between LFOs (delta, theta) and HFOs (gamma) of the V1 after 4-days MD during juvenile and adulthood were tested by evaluating the PAC of respective oscillations, and mean modulation index (MI) was calculated (Table 1).

In adulthood 4 days MD, a phase-amplitude coupling between delta-gamma was observed (Table 1). To further investigate the effect of MD on neuronal oscillatory synchrony in the ipsilateral eye (IE) and contralateral eye (CE), MI was compared between the eyes. No significant difference in MI±SEM was observed between IE vs. CE (delta-gamma, IE vs. CE, 4.5±0.40 vs 4.5±0.52, $p>0.05$). Similarly, no significant difference in MI±SEM was observed between IE vs. CE (theta-gamma, IE vs. CE, 0.05±0.00 vs 0.05±0.00, $p>0.05$). Plasticity in V1 was seized in adulthood, and it was not altered by the MD of one eye.

On the contrary, in CP 4 days MD, a phase-amplitude coupling between delta-gamma and theta-gamma were observed (Table 1). The effect of MD on neuronal oscillatory synchrony was further investigated in the IE and CE. A significant difference in MI±SEM was observed between IE vs CE (delta-gamma, IE vs. CE, 6.63±0.4 vs. 4.72±0.22, $p<0.01$; theta-gamma, IE vs. CE, 4.59±0.03 vs. 2.54±0.02, $p<0.01$). Note, MD during the CP leads to the neuroplasticity changes in the visual cortex. CPMD leads to the shift in the eye response towards the IE. Our results of coupling between delta-gamma and theta-gamma provide an insight into neuronal oscillatory synchrony of visual cortex neurons during CPMD.

Our above finding provides an understanding of the role of oscillatory rhythms in the low and high-frequency range in establishing precise synchronization of neuroplasticity changes in the V1 following MD.

B. The possible cross-frequency interactions of low-frequency bands with different gamma bands

To further investigate the effect of MD we measured the coupling of delta and theta with different gamma bands of the V1. MI was calculated across 8 different frequency combinations low frequency (delta, theta) and high-frequency gamma1 (30-55Hz), gamma2 (60-115Hz), gamma3 (125-175 Hz), and gamma4 (185-300Hz) was compared between IE and CE (Fig. 2). In adulthood 4 days MD, firstly the MI±SEM of IE was compared with the CE between delta with gamma1 (IE vs. CE, 1.12±0.15 vs. 1.10±0.13, $p>0.05$), gamma2 (IE vs. CE, 0.30±0.00 vs. 0.34±0.00, $p>0.05$), gamma3 (IE vs. CE, 0.17±0.00 vs. 0. 18±0.00, $p>0.05$) and gamma4 (IE vs. CE, 0.21±0.00 vs. 0.17±0.00, $p>0.05$). No significant difference in MI was observed between IE when compared with CE. Secondly, the MI±SEM of IE was compared with the CE between theta with gamma1 (IE vs. CE, 0.04±0.00 vs. 0.06±0.00, $p>0.05$), gamma2 (IE vs. CE, 0.05±0.00 vs. 0.02±0.00, $p>0.05$), gamma3 (IE vs. CE, 0.05±0.00 vs. 0.09±0.00, $p>0.05$) and gamma4 (IE vs. CE, 0.03±0.00 vs. 0.05±0.00, $p>0.05$). No significant difference in MI was found between IE when compared with CE.

In CP 4 days MD mice, firstly the MI±SEM of the IE was compared with the CE between delta with gamma1 (IE vs. CE, 5.81±0.41 vs. 3.93±0.37, $p<0.01$), gamma2 (IE vs. CE, 9.35±0.67 vs. 7.31±0.37, $p<0.01$), gamma3 (IE vs. CE, 8.14±0.51 vs. 5.57±0.25, $p<0.01$) and gamma4 (IE vs. CE, 3.22±0.18 vs. 2.70±0.18, $p<0.05$). The MI of the delta with all gamma bands was significantly higher in the IE when compared to the CE. Secondly, the MI±SEM of the IE was compared with the CE between theta with gamma1 (IE vs. CE, 1.35±0.02 vs. 0.83±0.01, $p>0.05$), gamma2 (IE vs. CE, 6.11±0.03 vs. 3.86±0.00, $p<0.001$), gamma3 (IE vs. CE, 6.32±0.03 vs. 2.93±0.00, $p<0.001$) and gamma4 (IE vs. CE, 0.17±0.01 vs. 0.73±0.06, $p>0.05$). The MI of theta with medium gamma and high gamma was significantly higher in IE when compared with the CE (Fig.2).

Our results demonstrate the experience-dependent plasticity induced by CPMD was characterized by the change in the coupling between low and high-frequency synchrony, but not in adulthood MD.

Groups	Mean Modulation index (MI±SEM) (delta-gamma)			Mean Modulation index (MI±SEM) (theta-gamma)		
	CE	IE	*p*-value	CE	IE	*p*-value
WT Adult MD (P60-P65)	4.50± 0.40	4.55± 0.52	n.s	0.05± 0.00	0.05± 0.00	n.s
Juvenile CPMD (P23-P28)	4.72± 0.22	6.63± 0.40	<0.01	2.54± 0.02	4.59± 0.03	<0.01

Table 1. Effect of monocular deprivation in adulthood and juvenile on the delta–gamma and theta-gamma mean modulation index of C57BL/6 mice. No significant (ns)($p>0.05$) difference is in the contralateral eye (CE) and ipsilateral eye (IE) in adulthood MD. A significant difference ($p<0.01$) between CE and IE in juvenile MD. Mean modulation index (MI) is normalized by 10^{-3} and expressed as MI±SEM.

IV. DISCUSSION

On a neuronal time scale, the activity within and across nerve cell populations is coordinated and integrated by the rhythmic synchronization of neuronal activity [18]. PAC plays a key role in the detection of sensory signals (perceptual), cognitive and sensorimotor processing [3]. In a recent study, delta-gamma coupling has been studied in the V1 and found that higher modulation in frequencies occurs when broadband naturalistic inputs are presented [19]. Visual brain stimulation provides functional evidence for the theta-gamma neuronal code in memory formation processes [20]. Dysfunctional coupling or synchrony is used as a biomarker of several neurodegenerative and neurological diseases such as Epilepsy, Parkinson's disease, Autism, and Alzheimer's disease [21, 22]. In the present study, we have explored the neuronal synchrony in the amblyopia mouse model. The results showed that 4 days of CPMD enhances the phase-amplitude of neural oscillations. Significant enhancement of PAC of the delta with all gamma bands was observed in IE as compared to the CE ($p<0.01$). Also, the PAC of theta with medium and high gamma was observed in IE as compared to the CE ($p<0.001$). In contrast, 4 days' adulthood MD did not show any significant ($p>0.05$) enhancement of PAC between delta-gamma and theta-gamma oscillations. Our results suggest that PAC exhibits different mechanisms for neuronal processing in juvenile MD and adulthood MD, indicating PAC may represent a potential neural dynamic marker for CP

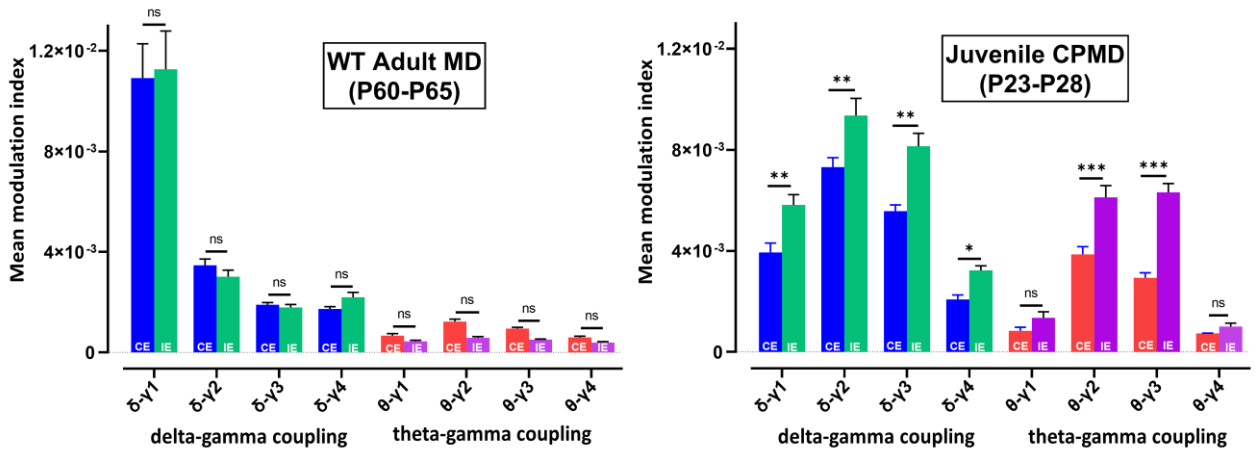

Fig. 2. Effect of monocular deprivation in adulthood and juvenile across 8 different frequency combinations between low frequency (delta (0.5-5Hz) & theta(5-10Hz)) and high frequency (gamma1 (30- 55Hz), gamma2 (60-115Hz), gamma3 (125-175Hz) and gamma4 (185-300 Hz)) of C57BL/6 mice. No significant (ns) (p>0.05) difference in the contralateral eye (CE) and ipsilateral eye (IE) in adulthood MD on delta-gamma (1-4) and theta-gamma (1-4). In juvenile MD, a significant difference (p<0.001) is observed between CE and IE on theta-gamma2 and theta-gamma3, (p<0.01) is observed between CE and IE on delta-gamma1, delta-gamma2, delta-gamma3 and (p<0.05) is observed between CE and IE on delta-gamma4. No significant (ns) (p>0.05) difference in the CE and IE on theta-gamma1 and theta-gamma4. Error bars in this study indicate standard error of the mean (SEM).

plasticity. The experience-dependent plasticity induced by CPMD was characterized by the change in the coupling between low and high-frequency synchrony, between IE and CE. Our results demonstrate an enhanced delta-gamma and theta-gamma coupling after CPMD during adolescence, indicating a broad range of nested oscillatory markers that are inherent to neuronal processing and are very consistent with the hypothesized increase in intrinsic coupling arising from neural oscillatory phase alignment. Thus, the use of LFP signals in the present study to detect changes in the strength of PAC across different states assured us that oscillatory activity plays an active role in enabling circuit plasticity of the visual cortex.

V. Conclusion

Mismatch in the input from the eyes during CPMD may activate ensembles of neuronal cells in a highly synchronous manner, giving rise to strong delta-gamma and theta-gamma oscillation driving the plasticity process. Our results demonstrate the high-frequency activity coupled to low-frequency phase synchrony during CPMD. For future work, other neural ensembles feature such as coherency between IE vs. CE in WT adult MD and CPMD animals will be further explored.

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References

[1] R. T. Canolty and R. T. Knight, "The functional role of cross-frequency coupling," *Trends Cogn Sci,* vol. 14, no. 11, pp. 506-15, Nov 2010.

[2] G. Thut, C. Miniussi, and J. Gross, "The functional importance of rhythmic activity in the brain," *Curr Biol,* vol. 22, no. 16, pp. R658-63, Aug 21 2012.

[3] M. Koster, H. Finger, S. Graetz, M. Kater, and T. Gruber, "Theta-gamma coupling binds visual perceptual features in an associative memory task," *Sci Rep,* vol. 8, no. 1, p. 17688, Dec 6 2018.

[4] M. Bonnefond, S. Kastner, and O. Jensen, "Communication between Brain Areas Based on Nested Oscillations," *eNeuro,* vol. 4, no. 2, Mar-Apr 2017.

[5] M. Bonnefond and O. Jensen, "Gamma activity coupled to alpha phase as a mechanism for top-down controlled gating," *PLoS One,* vol. 10, no. 6, p. e0128667, 2015.

[6] P. Lakatos, A. S. Shah, K. H. Knuth, I. Ulbert, G. Karmos, and C. E. Schroeder, "An oscillatory hierarchy controlling neuronal excitability and stimulus processing in the auditory cortex," *J Neurophysiol,* vol. 94, no. 3, pp. 1904-11, Sep 2005.

[7] J. M. Palva, S. Palva, and K. Kaila, "Phase synchrony among neuronal oscillations in the human cortex," *J Neurosci,* vol. 25, no. 15, pp. 3962-72, Apr 13 2005.

[8] S. Katzner, I. Nauhaus, A. Benucci, V. Bonin, D. L. Ringach, and M. Carandini, "Local origin of field potentials in visual cortex," *Neuron,* vol. 61, no. 1, pp. 35-41, Jan 15 2009.

[9] G. Spyropoulos, C. A. Bosman, and P. Fries, "A theta rhythm in macaque visual cortex and its attentional modulation," *Proc Natl Acad Sci U S A,* vol. 115, no. 24, pp. E5614-E5623, Jun 12 2018.

[10] D. Thengone, K. Gagnidze, D. Pfaff, and A. Proekt, "Phase-Amplitude Coupling in Spontaneous Mouse Behavior," *PLoS One,* vol. 11, no. 9, p. e0162262, 2016.

[11] A. Antonini and M. P. Stryker, "Plasticity of geniculocortical afferents following brief or prolonged monocular occlusion in the cat," *J Comp Neurol,* vol. 369, no. 1, pp. 64-82, May 20 1996.

[12] K. K. Lensjo, M. E. Lepperod, G. Dick, T. Hafting, and M. Fyhn, "Removal of Perineuronal Nets Unlocks Juvenile Plasticity Through Network Mechanisms of Decreased Inhibition and Increased Gamma Activity," *J Neurosci,* vol. 37, no. 5, pp. 1269-1283, Feb 1 2017.

[13] V. S. Sohal, F. Zhang, O. Yizhar, and K. Deisseroth, "Parvalbumin neurons and gamma rhythms enhance cortical circuit performance," *Nature,* vol. 459, no. 7247, pp. 698-702, Jun 4 2009.

[14] M. Bartos, I. Vida, and P. Jonas, "Synaptic mechanisms of synchronized gamma oscillations in inhibitory interneuron networks," *Nat Rev Neurosci,* vol. 8, no. 1, pp. 45-56, Jan 2007.

[15] S. Chauvette, S. Crochet, M. Volgushev, and I. Timofeev, "Properties of slow oscillation during slow-wave sleep and anesthesia in cats," *J Neurosci,* vol. 31, no. 42, pp. 14998-5008, Oct 19 2011.

[16] R. Fiath *et al.*, "Laminar analysis of the slow wave activity in the somatosensory cortex of anesthetized rats," *Eur J Neurosci,* vol. 44, no. 3, pp. 1935-51, Aug 2016.

[17] A. B. Tort, R. Komorowski, H. Eichenbaum, and N. Kopell, "Measuring phase-amplitude coupling between neuronal oscillations of different frequencies," *J Neurophysiol,* vol. 104, no. 2, pp. 1195-210, Aug 2010.

[18] A. K. Engel, P. Fries, and W. Singer, "Dynamic predictions: oscillations and synchrony in top-down processing," *Nat Rev Neurosci,* vol. 2, no. 10, pp. 704-16, Oct 2001.

[19] A. Mazzoni, K. Whittingstall, N. Brunel, N. K. Logothetis, and S. Panzeri, "Understanding the relationships between spike rate and delta/gamma frequency bands of LFPs and EEGs using a local cortical network model," *Neuroimage,* vol. 52, no. 3, pp. 956-72, Sep 2010.

[20] M. Koster, U. Martens, and T. Gruber, "Memory entrainment by visually evoked theta-gamma coupling," *Neuroimage,* vol. 188, pp. 181-187, Mar 2019.

[21] P. Bazzigaluppi *et al.*, "Early-stage attenuation of phase-amplitude coupling in the hippocampus and medial prefrontal cortex in a transgenic rat model of Alzheimer's disease," *J Neurochem,* vol. 144, no. 5, pp. 669-679, Mar 2018.

[22] Y. Salimpour and W. S. Anderson, "Cross-Frequency Coupling Based Neuromodulation for Treating Neurological Disorders," *Front Neurosci,* vol. 13, p. 125, 2019.