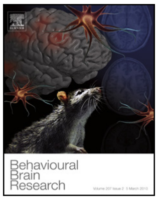
Behavioural Brain Research 265 (2014) 121–131

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr



Research report

Predictive nature of prefrontal theta oscillation on the performance of trace conditioned eyeblink responses in guinea pigs

Hao Chen[a], Yi-jie Wang[a,c], Li Yang[a], Chen Hu[a,d], Xian-feng Ke[a,d], Zheng-li Fan[a], Jian-feng Sui[a,b,*], Bo Hu[a,*]

[a] Department of Physiology, College of Basic Medical Sciences, Third Military Medical University, Chongqing 400038, PR China
[b] Experimental Center of Basic Medicine, College of Basic Medical Sciences, Third Military Medical University, Chongqing 400038, PR China
[c] Battalion 5 of Cadet Brigade, Third Military Medical University, Chongqing 400038, PR China
[d] Battalion 8 of Cadet Brigade, Third Military Medical University, Chongqing 400038, PR China

HIGHLIGHTS

- We assessed the impact of spontaneous mPFC theta oscillation on TEBC.
- Stronger baseline mPFC theta oscillations predicted more adaptive performance of CRs.
- However, the predictive effect was time-limited to the well-learning stage.
- Additionally, the prediction was specific to TEBC with a trace interval of 500 ms.

ARTICLE INFO

Article history:
Received 4 February 2014
Received in revised form 11 February 2014
Accepted 15 February 2014
Available online 23 February 2014

Keywords:
Theta oscillation
Medial prefrontal cortex
Trace conditioning
Eyeblink
Guinea pig

ABSTRACT

Stimulus-evoked theta oscillations are observed in the medial prefrontal cortex (mPFC) when executing a variety of learning tasks. Here, we aimed to further determine whether spontaneous theta-band (5.0–10.0 Hz) oscillations in the mPFC predicted the subsequent behavioral performance in trace eyeblink conditioning (TEBC), in which the conditioned stimulus (CS) was separated from the unconditioned stimulus (US) by 500 ms trace interval. By recording local field potentials (LFP) signals in the guinea pigs performing the TEBC task, we found that, a higher mPFC relative theta ratio [theta/(delta + beta)] during the baseline (850-ms period prior to the onset of the CS) was predictive of higher magnitude and more adaptive timing rather than faster acquisition of trace conditioned eyeblink responses (CR). However, the prediction of baseline mPFC theta activity was time-limited to the well-learning stage. Additionally, the relative power of mPFC theta activity did not correlate with the CR performance if the trace interval between the CS and the US was shortened to 100 ms. These results suggest that the brain state in which the baseline mPFC theta activity is present or absent is detrimental for the subsequent performance of trace CRs especially when the asymptotic learning state is achieved.

1. Introduction

Trace eyeblink conditioning (TEBC) is an experimentally tractable behavioral model of associative motor learning. The primary memory trace of conditioned eyeblink responses (CR) acquired during TEBC is hypothesized to locate in the cerebellum and associated brainstem circuitry [1–7]. However, to bridge a long trace interval (e.g., 250 ms for mice [8] and rats [9]; 500 ms for rabbits [10], guinea pigs [11], and human beings [12] between the conditioned stimulus (CS: e.g., a pure tone) and the unconditioned stimulus (US: e.g., an airpuff directed to the corneal), several forebrain areas are recruited to support and/or facilitate the learning process (for reviews, see [13–16]).

One of the forebrain areas implicated in TEBC is the medial prefrontal cortex (mPFC). A line of evidence supporting this notion stems from that lesion/inactivation made in the mPFC disrupts the learned responses [2,17,18]), and also prevents learning in the animals that are inactivated before the CRs are acquired [19–22]. Additional evidence comes from the extracellular electrophysiological recording studies which show learning-

---

[*] Corresponding authors at. Department of Physiology, College of Basic Medical Sciences, Third Military Medical University, Gaotanyan Street 30, Shapingba District, Chongqing 400038, PR China. Tel.: +86 23 68752253 0/+86 23 68753704..

E-mail addresses: jfsui2003@163.com (J.-f. Sui), bohu@tmmu.edu.cn, bhu_tmmu@163.com (B. Hu).

http://dx.doi.org/10.1016/j.bbr.2014.02.020
0166-4328/

related neuronal activity in the mPFC when performing the TEBC task [23–26]. Moreover, the mPFC is revealed to be involved in the consolidation [27] and the retrieval [28] of trace CRs. Overall, these findings show highlights on the involvement of mPFC in TEBC.

Theta oscillations have been demonstrated to provide important neural mechanisms for linking the prefrontal cortex with the hippocampus, another forebrain area critically involving in TEBC [8,10–12], during a variety of learning tasks [29–33]. Therefore, recent interests begin to focus on understanding the role of prefrontal theta oscillations during TEBC. For example, it has been shown that the phase of CS-induced theta oscillations in the mPFC couples with the amplitude of local gamma oscillations, which is proposed to result in stronger prefrontal neuronal responses to the CSs [34]. Moreover, the CS-evoked theta oscillations in the mPFC are revealed to synchronize with those in the entorhinal cortex, which may contribute to the consolidation of trace CRs [35]. These observations indicate that the CS-evoked theta oscillations in the mPFC are critically involved in TEBC. Intriguingly, in a trace conditioning task, the spontaneous mPFC theta oscillations were observed during the stimulus-free epochs of quiet waking [36]. To date, questions remain about the potential benefit of these spontaneous mPFC theta oscillations for TEBC. The interest of this issue derived from earlier data that the spontaneous hippocampal theta oscillations strongly correlated with the acquisition of trace CRs [37,38]. In parallel, the theta-contingent trial CS presentations accelerated the acquisition rate during TEBC [39]. Consequently, the current study aimed further to assess the effect of spontaneous mPFC theta activity on the subsequent acquisition and/or performance of trace CRs. Given that there is a gradual transfer of transient hippocampus-dependent memory of trace CRs to the distributed neocortical sites (e.g., mPFC) as conditioning progresses [2,40,41], we expect to observe that the spontaneous mPFC theta oscillations are connected to the CR acquisition and/or performance in the late, rather than the early stage of learning.

2. Materials and methods

2.1. Subjects

The subjects were adult male albino Dunkin-Hartley guinea pigs weighing between 450 and 550 g (3–4 months old) in this experiment. All the animals were individually housed and had free access to food and water in their home cages. All the experimental procedures were approved by the Animal Care Committee of the Third Military Medical University and were performed in accordance with the principles outlined in the NIH guide for the care and use of laboratory animals.

2.2. Electrode implantation

The detailed implantation procedures were previously described by Chen et al. [42]. In brief, each animal was implanted with a monopolar stainless steel electrode (NO. 792300, A-M systems, Carlsberg, WA, USA). The electrode (bare diameter: 254.0 μm, covered diameter: 330.2 μm) was implanted into the right mPFC (1.0 mm lateral to midline suture, 2.0 mm ventral to dura, and 2.0 mm anterior to Bregma) according to a brain atlas of the guinea pig [43]. Four stainless steel screws then were positioned on the surface of skull to serve as reference (2.5 mm posterior to Bregma, ±5.0 mm lateral to midline suture; 8.5 mm posterior to Bregma, ±5.0 mm lateral to midline suture). Besides, the animals were fitted with a headstage and a loop attached to the apex of the left upper eyelid. As described by Hu et al. [44], this loop was used to attach the left upper eyelid to a high- resolution movement-measuring device.

2.3. Behavioral training

After one week of postoperative recovery, the animals were placed in a Plexiglas containing box located in a sound- and light-attenuating chamber for 2 days, 30 min per day. These measurements were utilized for the animals to familiarize the experimental situation. Thereafter, the adapted animals were given TEBC training as described by Hu et al. [45]. The CS was a tone (2 kHz, 85 dB, 350 ms, and 5 ms rise/fall time), which was paired with a left corneal airpuff US (100 ms, 3.0 psi measured at the source). The CS offset was separated by 500 ms (or 100 ms) from the US onset. Conditioning training consisted of 5 blocks of 10 CS-US pairings trials for a total of 50 trials per session per day. The inter-trial interval varied from 20 to 40 s with a mean value of 30 s, and the animals were consecutively trained for nine days. As a control, the pseudorandomized animals received an equal number of CSs and USs but never in the explicitly temporal sequence that would induce learning.

2.4. Recordings

The LFP signals were measured as the voltage difference between the monopolar recording electrode and the reference screws placed above the skull, and were amplified by 1000 times and band-pass filtered (0.5–30 Hz). The LFP signals, eyelid movement mechanogram and markers of the applied stimuli were digitized at a sample rate of 10 kHz by a data acquisition system (Powerlab 8/35, ADInstuments, Australia) and were acquired using the software Chart (v. 7.0).

2.5. Data analysis

2.5.1. Eyeblinks

Detailed description of eyeblink response analysis was previously described [11]. In brief, for each trace conditioning trial, we calculated the average above-threshold activity for the baseline (1–350 ms period before the CS onset), startle eyeblink response (SR: 1–120 ms period after the CS onset), conditioned eyeblink response (CR: 121–850 ms or 121–450 ms period after the CS onset), and unconditioned eyeblink response (UR: 1–500 ms period after the US onset). A significant eyelid movement was defined as an increase in the integrated activity that was greater than the mean baseline activity plus four times standard deviation (SD) of the baseline activity. In addition, an eyelid movement required to have a minimal duration of 15 ms and had to exceed the 1.0 mV threshold (equaled 0.25 mm). Any significant eyelid movement during the period mentioned above was counted as a SR, a CR or an UR, respectively. Nine parameters were used to evaluate the acquisition or the performance of eyeblink responses: (1) CR incidence; (2) CR peak amplitude; (3) CR peak latency; (4) CR onset latency; (5) UR peak amplitude; (6) UR peak latency; (7) SR peak amplitude; (8) SR peak latency; and (9) SR onset latency.

To assess the impact of prefrontal theta activity on the CR performance, two separate learning stages from each animal were selected for analysis: one stage with CR incidence of ~50%, representing the middle-learning state (MLS, days 4–5), and the other stage with the CR incidence ~75%, representing the well-learning state (WLS, days 8–9). Similar to the consideration of Nokia et al. [46], selection of these particular stages was explained by the relatively large numbers of trials showing a CR related to make the comparison of averages recorded in the theta and nontheta trials. Additionally, the peak amplitude and the peak