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Phase–amplitude coupling between theta and gamma oscillations during nociception in rat electroencephalography

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ABSTRACT

In electroencephalography (EEG) study, gamma oscillations were reported to participate in pain processing; theta oscillations were also involved in pain processing. Moreover, theta always modulated gamma activity by phase–amplitude coupling in event-related oscillations. Whether theta modulate gamma by phase–amplitude coupling in pain processing is of interest. In the present study, using EEG of rats after laser nociceptive stimulation, we investigated gamma activity and phase–amplitude coupling between theta and gamma. It was found that induced gamma power increased starting 200 ms after nociceptive stimulation onset. Moreover, significant coupling between theta phase and gamma amplitude was found over frontal and parietal region after nociceptive stimulation. Our results for the first time suggest that coupling between theta and gamma is involved in nociception processing.

Oscillatory activities in the gamma frequency band (30–70 Hz) of electroencephalography (EEG) were considered to be a cortical representation of processing sensory information such as visual [9,28] and audio [8] information. Gamma oscillations play an important role in visual and audio perception by binding several features of the stimulus to form coherent perception [9,11,14]. Recently, gamma oscillations have been found to be involved in the processing of nociceptive information in a magnetoencephalography (MEG) study. In EEG studies, it was also found that induced gamma oscillatory activity increased after nociceptive stimulation [6,10]. Interestingly, induced gamma activities were stronger in perceived stimuli than in unperceived stimuli. These reports indicate that gamma oscillations are involved in pain perception. In addition to fast gamma oscillations, oscillations in low theta frequency band (4–8 Hz or 3–7 Hz) also participate in perceptional tasks [4] as well as in pain perception [21].

Previous studies found that the amplitude (or power) of the gamma oscillations was systematically modulated by the phase of theta oscillations in both rats and human beings [3,5]. For example, during an auditory task, the amplitude of gamma oscillation was modulated by the phase of theta oscillation in stimulus-induced activity from auditory cortex [15]. This observed cross-frequency coupling between theta phase and gamma amplitude was considered to be important in processing sounds [15]. In addition, during visual perception, gamma amplitudes were also coupled to theta phase in human EEG [7,27].

Based on these reports, it seems plausible that there is interaction between theta phase and gamma amplitude during the processing of pain information. Therefore, we propose that amplitude of gamma oscillations is modulated by phase of theta oscillations through phase–amplitude coupling in pain perception.

In the present study, we exploited cortical EEGs from behaving rats to study whether there is coupling between theta phase and gamma amplitude after perceived nociceptive stimulation. Gamma activities induced by perceived laser nociceptive stimulation and the cross-frequency coupling between theta phase and gamma amplitude were investigated. It was found that the amplitude of gamma oscillations was coupled to the phase of theta oscillations after perceived nociceptive stimulation.

Eight Sprague-Dawley adult rats were used in this experiment. They were provided by the Department of Experimental Animal Sciences, Peking University Health Science Center. They were housed individually in cage with the temperature maintained at $22 \pm 1\,^{\circ}C$ and kept under a natural light/dark cycle. Food and water were available *ad libitum*. Rats were habituated to the environment and handled daily for one week before electrode implantation surgery.

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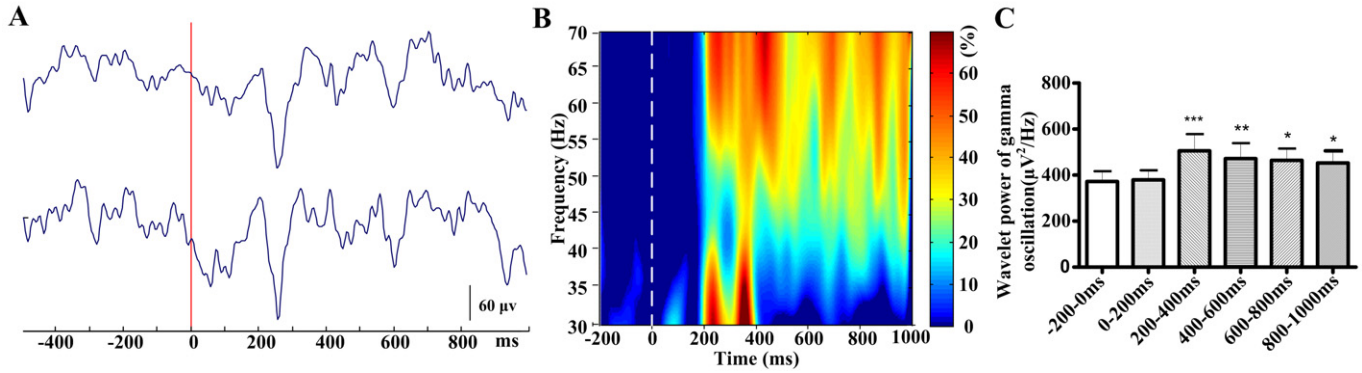

Fig. 1. Gamma power increased 200–1000 ms after laser stimulation. (A) An example of cortical EEG induced by CO_2-laser stimulation in two channels. The vertical line indicates the time of laser stimulation. (B) Change of gamma oscillation power before and after laser stimulation averaged cross all channels in rats. (C) Gamma power between 0 and 200, 200 and 400, 400 and 600, 600 and 800 ms, and 800 and 1000 ms was compared with that of baseline between −200 and 0 ms. $n = 11$. $^{***}P < 0.001$, $^{**}P < 0.01$, and $^{*}P < 0.05$ (repeated ANOVA with Dunnett's multiple comparison test).

All animal experimental procedures were conducted in accordance with the guidelines of the International Association for the Study of Pain [29] and were approved by the Animal Care and Use Committee of Peking University.

Rats were anesthetized with sodium pentobarbital (50 mg/kg, *i.p.*). After we removed the scalp of the rat and exposed the skull, fourteen stainless steel screws (tip diameter 1 mm, impedance 300–350 Ω) with sockets were implanted as epidural electrodes into the skull to record cortical EEGs. The location of these electrodes was determined by the method from Shaw et al. [22]. These electrodes were fixed to the skull with dental cement and they were not connected with any muscles. Penicillin (60,000 U, *i.m.*) was administrated for 3 days to prevent possible infection.

Rats were habituated in the recording cage for 30 min, then they were stimulated with a laser beam (wavelength 10.6 μm, beam diameter 2.5 mm, pulse width 20 ms) to the hindpaw delivered by a CO_2-laser stimulator (DIMEI-300, Changchun Optics Medical Apparatus Co., Ltd., China). The energy level of the laser beam for an individual rat was determined by a series of laser beams with ascending energy. The energy level for which 4–5 hindpaw withdrawal responses were generated out of 6 stimuli was chosen as the energy level for each rat. Stimuli were given when the rat was awake and quiet. Each stimulus was targeted to a slightly different position to avoid sensitization, and the stimulus interval was bigger than 40 ms. A total of 15 thermal stimuli with hindpaw withdrawal responses at this energy level were collected in each rat for further EEG data analysis. EEG recordings with a sampling frequency of 1024 Hz were carried out during laser stimulation. The EEG/ERP system (CogniTrace ERP, ANT Inc., The Netherlands) was used for data collection.

After movement artifact or large baseline drift were removed from all trials and channels, the EEG signals were re-referenced to an average of all channel recordings. EEG data from 500 ms before to 1000 ms after the laser stimulation onset (totally 1500 ms) was defined as an epoch.

The wavelet power spectrum was used to obtain the power of gamma-frequency oscillation (30–70 Hz) for each epoch [16]. The time–frequency analysis of every epoch was subjected to the above wavelet decomposition with 1 Hz increment yielding a power × time × frequency matrix for each trial. Trial-averaged power matrix was computed for laser-induced gamma activity (non-phase-locked activity) of each channel [1]. The change of power was expressed as a percentage relative to power of baseline (−200 to 0 ms prior to the stimulation onset).

After high frequency $x_H(n)$ (30–70 Hz) and low frequency $x_L(n)$ (3–20 Hz) components were obtained by FIR filter (eegfilt.m from the EEGLAB toolbox), the Hilbert transformation was used to get their amplitudes and phases for each epoch. According to phase of low frequency φ_L and amplitude of high frequency a_H, we constructed $[\varphi_L(n), a_H(n)]$ to get the amplitude information of each phase and estimated the phase amplitude coupling (PAC) with the method proposed by Tort et al. [26]. Then, 100 surrogate data generated by trial were shuffled to obtain the significance of coupling. The significant PAC whose $P < 0.05$ were retained while the PAC whose $P > 0.05$ was set to 0.

We used one-way repeated measures analysis of variance (one-way repeated ANOVA) with time as repeated factor to test the significance of changes of induced gamma activity after laser stimulation compared with baseline. One-way ANOVA was applied for comparing the coupling between gamma–theta PAC and gamma–alpha (8–12 Hz), gamma–beta1 (12–16 Hz), gamma–beta2 (16–20 Hz) PAC. Post comparisons were performed with Dunnett's test to compare gamma oscillation before and after stimulation and to compare gamma-other frequency PACs with the gamma–theta PAC.

The power of gamma frequency oscillation from −200 ms to 1000 ms was calculated in each epoch of all channels. The grand averaged power change of gamma-frequency oscillation among all channels is shown in Fig. 1B. After nociceptive laser stimulation onset, the power in the gamma frequency band increased compared with that before stimulation onset. As shown in Fig. 1B, increase of gamma power was distinct especially between 30 and 40 Hz during 200–400 ms and between 50 and 70 Hz from 200 ms after stimulation onset. To characterize the time course of gamma power changes, we compared the gamma power in each period of time. The statistical results with one-way repeated ANOVA showed that the gamma power in the period of 0–200 ms was the same as baseline, while power in other periods after 200 ms was significantly increased ($F = 6.068$, $P < 0.005$) (Fig. 1C). These results suggest that nociceptive stimulation could enhance gamma power beginning 200 ms after stimulation onset.

In order to test whether this increased gamma oscillation was coupled to oscillations in theta band or in other frequency bands, we studied the coupling between the phase of low frequency (3–20 Hz) oscillation and the amplitude of high frequency (30–70 Hz) gamma oscillation from 0 to 1000 ms after nociceptive stimulation from each epoch of all channels. As shown in Fig. 2, only for frequencies below 7 Hz was the phase of oscillations obviously coupled to the amplitude of gamma frequency oscillation (Fig. 2A). The strength of coupling was more evident between oscillations in 3–5 Hz and 30–50 Hz. Statistical analysis showed that the amplitude of gamma frequency oscillations (30–70 Hz) was significantly coupled to the phase of theta oscillations (3–7 Hz) rather than other frequency oscillations ($F = 539.1$, $P < 0.0001$) (Fig. 2B). We also studied the

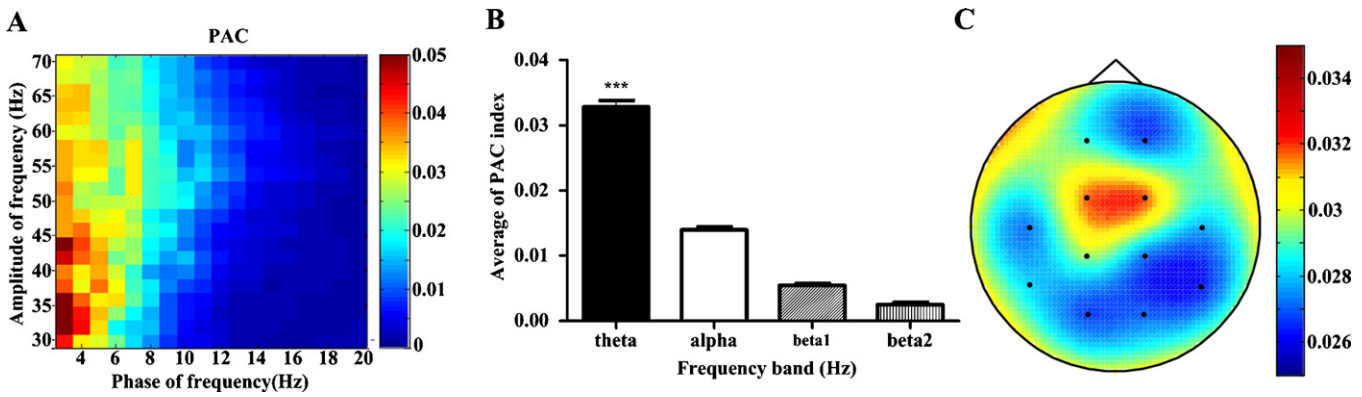

Fig. 2. Amplitude of gamma oscillation is modulated by phase of theta oscillation after laser stimulation. (A) Averaged phase–amplitude coupling (PAC) between low frequency (3–20 Hz) and high frequency (30–70 Hz) among all channels in rats. (B) Phase–amplitude coupling between gamma oscillation (40–70 Hz) and theta (3–7 Hz), alpha (8–12 Hz), beta1 (12–16 Hz), beta2 (16–20 Hz). Phase–amplitude coupling between theta and gamma oscillation was significantly stronger than coupling between other oscillations and gamma oscillation. $^{***}P < 0.001$. (C) Topography of phase–amplitude coupling between theta and gamma oscillations.

topography distribution of the phase–amplitude coupling between theta and gamma oscillations. It was found that the coupling was mainly located at the parietal region (Fig. 2C). All these results indicated an increased synchronization between theta phase and gamma amplitude over frontal and parietal region after nociceptive stimulation.

In this study, we found that the gamma activity increased beginning 200 ms after nociceptive stimulation, and the amplitude of gamma was coupled to the phase of theta frequency oscillations during pain processing.

It is known that not all nociceptive stimuli with same intensity above threshold could elicit nociceptive hindpaw withdraw responses of rat. In our present study, the stimuli selected in data analysis were those stimuli that could elicit nociceptive hindpaw withdraw responses. Nociceptive hindpaw withdraw response is a reflection of nociception, so, what our study focused on was oscillatory activities of nociception processing. The present study found that induced gamma activities increased by stimuli that elicited nociception behavior (Fig. 1). A pervious MEG study reported that laser nociceptive stimulation could increase the induced gamma activity in primary sensory cortex; furthermore, this increased induced-gamma activity was positively correlated with pain perception. Therefore, our result, in line with the previous report [10], indicated that this enhanced gamma activity was involved in nociception processing.

Compared to electrical and mechanical stimulation which activate simultaneously A-beta, A-delta and C-fibers, CO_2 laser stimulation is a special and nociceptive-specific tool for activating pain-related A-delta and C-fibers [18]. In the local field potential (LFP) study, the reaction time at 250–400 ms was considered to be a reflection of A-delta activation. In the present study, with wavelet analysis of EEG of rats, we found that the induced gamma increased at 200–1000 ms after nociceptive stimulation (Fig. 1B). Based on the latency in our result, this oscillation is most likely due to A-delta activation. This is in line with previous studies that only A-delta fibers are activated in most laser evoked potentials.

Gamma activity emerging 200 ms after stimulation is regarded as "late gamma" which is considered to be related to storage and usage of sensory information [12,25]. It is well known that pain perception is information that could prevent further damage, so storage of pain information must be an important part of pain information processing. This induced late gamma activity in our result is possibly a reflection of neural processing of nociceptive information such as storage of pain information.

In the present study, we found that the amplitude of gamma was significantly coupled to the phase of theta oscillation rather than phase of oscillation at other frequency bands (Fig. 2A and B). Previous studies found that gamma amplitude was modulated by the phase of theta oscillation from spontaneous oscillations in both human beings and rats [3,20]. This kind of coupling was also found in event-related oscillations [7,15]. Our result provides evidence for the coupling between gamma and theta oscillation in pain-related oscillations. Gamma activity is assumed to be the reflection of local neural networks. In terms of perception, gamma oscillation itself is not sufficient for integrating all the distributed information required for nociception. From our present results, it was shown that gamma oscillation could work together with theta oscillation in nociception. It is known that theta oscillation regulates long range interaction, which could communicate among different brain regions responsible for perception. Therefore, from our results, the coupling between theta and gamma played a role in nociceptive information processing possibly by integrating several aspects of nociceptive information.

Primary somatosensory cortex is responsible for sensory aspects of pain and the anterior cingulate cortex is responsible for emotional aspects of pain. Both regions are critical regions for pain perception. Topography findings in our study showed that the increased phase–amplitude coupling of theta and gamma oscillations was located over frontal and parietal cortices (Fig. 2C). There are locations of the primary somatosensory cortex and the anterior cingulate cortex of rat. Therefore, it is reasonable to infer that the coupling between phase of theta and amplitude of gamma integrate sensory and emotional aspects of pain and is involved in the processing of pain perception or nociception. More interestingly, the synchronization of theta phase and gamma amplitude oscillations has been suggested to support maintenance of items in working memory [13,17]. Synchronization between theta and gamma oscillations after nociceptive stimulation may be a reflection of working memory of pain, which is consistent with results of the increased late gamma after stimulation. But, this needs further investigation.

Movement artifacts, like the hindpaw withdrawal reflex after nociceptive stimulation, may influence EEG activities in behaving rats. For example, it was reported that rodents showed increased oscillatory activity around 20 Hz with movement [2]. In our study, the frequencies of the oscillations were 30–70 Hz. Obviously, in our experiment, if movement artifacts had influenced the EEG activity, their effect should be reflected in lower frequencies like 30 Hz. To exclude the possibility that oscillation at lower frequencies (like 30–35 Hz in our experiment) was the major component, we also calculated the power and PAC of gamma at higher frequencies (40–70 Hz) and got a very similar result as with 30–70 Hz. In addition, some studies reported that movement did not show significant influence on cerebral electrical activities. For example, Sun et al. reported that the mean latency of the hind-paw withdrawal of rat

was around 282.8 ms which was similar to the latency of the laser evoked potential. However, the laser evoked sink source current remained unchanged after removal of movement (the rat was paralyzed) [24]. It was noted that the latency of our laser-related EEG was within the overlapping time-window of evoked sink source current. Furthermore, in moving rats, it was reported that high frequency activities (30–60 Hz) in the 250–750 ms increased after noxious laser stimulation compared with innocuous stimulation applied to the tail [23]. This indicated that gamma oscillation was more likely related to pain perception than to movement. Therefore, movement artifacts of the rat did not cause obvious interference with our results. In addition, the possible influence of electromyogram (EMG) artifacts could be largely prevented by our electrocorticography recordings. It is well known that the problem of EMG contamination is significant in scalp EEG recording but the EMG contamination is much less in electrocorticography recording. Further, a previous report found that the EMG contamination mainly appeared within 200 ms after laser stimulation [19]. So, the influence of EMG contamination in our results should be very small and could be ignored.

In the present study, with EEG analysis in rats, increased late induced gamma activity and the coupling between theta phase and gamma amplitude were found after nociceptive stimulation. This coupling may be a reflection of processing of nociceptive information in the cortex and is probably related to storage of pain information.

Conflict of interest

The authors declare no conflict of interest.

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