Gamma wave oscillation and synchronized neural signaling between the lateral hypothalamus and the hippocampus in response to hunger

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Abstract The lateral hypothalamus plays an important role in homeostasis. It is sensitive to negative energy balance and believed to interact with other brain regions to mediate food seeking behavior. However, no neural signaling of hunger in the lateral hypothalamus has been studied. Male Swiss albino mice implanted with intracranial electrodes into the lateral hypothalamus and the hippocampus were randomly treated with drinking water for control condition, 18-20 h deprivation of food for hunger condition, and fluid for satiety condition. Therefore, local field potential (LFP) and locomotor activity of animals were simultaneously recorded. One way ANOVA with Tukey’s post hoc test was used for statistical analysis. Frequency analysis of LFP revealed that food deprivation significantly increased the power of gamma oscillation (65-95 Hz) in the lateral hypothalamus and the hippocampus. However, satiety did not change the oscillation in these regions. Moreover, no significant difference among groups was observed for locomotor count and speed. The analysis of coherence values between neural signaling of these two brain areas also confirmed significant increase within a frequency range of 61-92 Hz for hunger. No change in coherence value was induced by satiety. In summary, this study demonstrated neural signaling of the lateral hypothalamus in response to hunger with differential power spectrum of LFP and the interplay with the hippocampus. The data may suggest critical roles of the lateral hypothalamus in detection of negative energy balance and coordination of other higher functions for food related learning or behaviors through the connectivity with the hippocampus.

Keywords Lateral hypothalamus · Hippocampus · Local field potential · Hunger · Energy balance

Introduction

A drive of eating behavior is one of crucial survival strategies. Physiologically, it is critical to maintain the energy balance through homeostatic processes. The detection of energy levels in the body by specific brain areas is highly sensitive to ensure the equivalence between energy intake and expenditure. For example, whenever negative energy balance is likely to progress, specific neuronal circuits are excited in a process to increase motivation that would trigger feeding behavior.

The lateral hypothalamus located on either side of the third ventricle has been considered as a key player in the regulation of food intake in mammals [1]. Bilateral destructions of this area in rats or cats led to complete inhibition of spontaneous eating [2]. This area is called the ‘feeding center.’ On the other hand, an increase in food intake has been induced by electrical stimulation of the lateral hypothalamus [3]. Similar responses were also induced by anticipation of food [4] or treatment with opioids agonist [5] or some glutamate agonist [6]. Moreover, blockade of excitatory amino acid receptors in the nucleus accumbens shell also exhibited feeding [7]. Therefore, this connection was hypothesized as the functional pathway between accumbens shell and the lateral hypothalamus. The study of neural bases of eating behavior has also been focused on the interactions between the lateral hypothalamus and other brain areas such as the nucleus accumbens and ventral pallidum in controlling feeding motivation [8]. This includes the connection with the hippocampus through the
hippocampal fibers reported to project to the lateral hypothalamus [9].

In reticulo-hypothalamic systems, a stimulation of the lateral hypothalamus was reported to affect the hippocampal theta oscillation [10]. Electrolytic lesion of the lateral hypothalamus activated aphagic mechanisms including the absence of normal atropine-resistant EEG activity in the hippocampus [11]. Changes in the hippocampal brain oscillation were also observed in response to stimulation or blockade of the lateral hypothalamus [12]. These findings appeared to confirm the connections between brain areas that some of them might function in association with feeding behaviors. However, the relationship between the lateral hypothalamus and the hippocampus in food intake mechanism remains unclear. In particular, the neural signaling and the interplay between these brain areas in association with feeding have not been studied.

This study aimed to investigate LFP oscillations in the lateral hypothalamus and the hippocampus of mice. LFP power spectrum in each brain areas and coherence between these two areas were evaluated during negative and positive energy balances in comparison to control energy levels.

Materials and Methods

Animals and electrode implantation

Fifteen male Swiss albino mice weighing 40-45 g were supplied by the Southern Animal Laboratory Facility of Prince of Songkla University, Songkhla, Thailand. Electrodes were implanted according to the procedures described by Cheha et al. [13]. Briefly, animals were anesthetized with a cocktail of ketamine and xylazine via intramuscular injection and positioned in a stereotaxic apparatus. Lidocaine was applied under the dorsal scalp as a local anesthesia to make midline incision. Therefore, intracranial electrodes were implanted on the left side of the brain to the lateral hypothalamus (anteroposterior (AP) to bregma -1.5 mm, mediolateral (ML) to bregma -1 mm, dorsoventral (DV) to bregma 5.2 mm), dorsal hippocampus CA1 (AP -2.5 mm, ML -1.5 mm, DV 1.5 mm) and cerebellum (AP -6.5 mm, Midline, DV 2 mm as a reference) according to mouse brain atlas [14]. Thereafter, electrodes were connected with the skull by using dental cement. The antibiotic ampicillin was given intramuscularly once a day for 3 days to prevent infection. Animals were allowed to recover for at least 2 weeks.

LFP data acquisition and data analysis

LFP signals and locomotor activity were recorded in a black cylinder chamber (33 cm diameter and 40 cm height). LFP signals were recorded by a PowerLab 16/35 system (AD Instruments, Australia) with 16-bit A/D, and stored in a PC computer through Lab Chart 7.3.7 Pro software. All LFP signals were processed through 1 - 200 Hz band-pass digital filter with a sampling frequency of 1 kHz. Recorded files were overviewed by using visual inspection and only noise-free signals were used for the analysis. 50 Hz notch filtering was applied to remove the power line noise. For data analysis, power spectral density (PSD) was generated by Lab Chart 7.3.7 Pro.

![Fig. 1](image1.png)  
**Fig. 1** Representative spectrograms of LFP signals from the lateral hypothalamus (a-c) and the hippocampus (d-f). Signals were recorded from animals in 3 conditions which included control (a and d), hunger (b and e) and satiety (c and f).
software using a Hanning window cosine (window size = 0.976 s, overlaps = 0.488 s). The PSD was calculated as the percent total power. The average spectral power was expressed in frequency domain (1 – 100 Hz) indicated for each condition. LFP coherence was analyzed using MATLAB R2012b (Version 8.0) expressed as the average of coherence value. Additionally, the software was used to analyze animal images detected via a video camera. The translocation of animal was caught with sensitivity at 2-mm threshold as previously described [13].

Experimental procedure

Baseline oscillation of the lateral hypothalamus and the hippocampus was obtained before the testing day. All mice were individually habituated in the test box for 30 min per day for 3 consecutive days. Prior to placing mice in the box, they were treated with an oral gavage (blank). On the testing day, control mice were put into the test box after feeding through gavage with 0.1 ml/10 g body weight of drinking water. For a group with hunger condition, animals with 18-20 h food deprivation were treated with blank gavage. For satiety condition, animals received an oral gavage with 0.1 ml/10 g body weight of relative to control both in the lateral hypothalamus (a) and the hippocampus (b).

Fig. 2 Percent total power in 1-100 Hz range are expressed as mean ± S.E.M. One-way ANOVA followed by Tukey’s post hoc test were used to indicate significant effect for hunger at 65-95 Hz relative to control both in the lateral hypothalamus (a) and the hippocampus (b).

Fig. 3 Locomotors activity of animals in 3 different conditions. Data are analyzed and expressed in terms of the number of locomotion (a) and velocity (b) using One-way ANOVA statistical analysis.
liquid food. Therefore, animals were individually placed in the box for EEG and locomotor activity recordings. Individual mice were assigned randomly for these 3 conditions in 3 consecutive days for repeated measures.

Statistical analysis

All data were averaged and expressed as mean ± Standard Error of Mean (S.E.M.). Changes in spectral power, locomotor activity and coherence values among 3 conditions were analyzed by using one-way ANOVA followed by Tukey’s post hoc method. P value < 0.05 was accepted to be statistically significant.

Results

Prior to frequency analysis of LPFs, signals were visually inspected for their general appearances. Power spectrograms of the signals from the lateral hypothalamus and the hippocampus during hunger and satiety period were shown with respect to that of control periods (Fig. 1). Obviously, different patterns between LFP spectrograms of the lateral hypothalamus and the hippocampus were seen. By using a gray scale code reference of power, the lateral hypothalamus appeared to have relatively lower power than that of the hippocampus. It could be seen by visual inspection that the hippocampus had a dominant activity of theta oscillation. However, it seemed unable to distinguish the difference among signals of different energy states within each brain area by using the spectrograms. Therefore, frequency analysis was performed to quantitate percent total power of the LFPs during a 20-25 min period in frequency domain. The results showed that lateral hypothalamus LFPs were characterized by the prominent peak within delta frequency band (2-5 Hz) (Fig. 1a-c) whereas hippocampal LFPs were characterized by the prominent peak within theta band (8-12 Hz) (Fig. 1d-f). One-way ANOVA revealed significant differences between lateral hypothalamus LFP powers during hunger condition and control condition in a range of 65-95 Hz [F (2, 44) = 3.300, P < 0.05] also known as gamma band (Fig. 2a). No change in lateral hypothalamus LFP was induced by satiety condition. Moreover, power spectrums of hippocampal LFPs were analyzed. Significant increase was induced by hunger in the same frequency range to that found in the lateral hypothalamus (Fig. 2b). By the way, satiety condition had no effect on hippocampal LFP oscillation.

Locomotor activity of animals during a 20-25 min period was analyzed in terms of number of locomotion and velocity. Values of hunger and satiety conditions were compared to control levels. Neither hunger nor satiety was found to have significant effect on these 2 parameters (Fig. 3).

The interplay between the lateral hypothalamus and the hippocampus was examined. Coherence values of LFP from these brain areas were analyzed for 1-100 Hz during a 20-25 min period. In comparison to control
values, significant increase was induced by hunger in a 61-92 Hz \([F (2, 44) = 5.747, P < 0.05]\) (Fig. 4). No significant change in coherence value was induced by satiety.

Discussion

Altogether, this study highlighted the application of signal analysis to understand neural processes of feeding. The confirmation of brain detection of negative energy balance in the lateral hypothalamus by LFP power spectrum was consistent with previous studies [3, 15]. The response found in the lateral hypothalamus was specific to hunger but not satiety. In general, the brain has been found to be involved in physiological mechanisms of energy homeostasis. Specific neural circuits in the lateral hypothalamus detect the alteration of energy deposits and expenditures to provide the optimized energy balance [4, 16].

However, an additional highlight proposed by the present study was the coherence between the lateral hypothalamus and the hippocampus arisen during a period of hunger. The coherence values hinted the active neuronal circuits for some homeostatic processes. In this study, the coherence analysis revealed the interplay between the lateral hypothalamus and the hippocampus following food deprivation for 18-20 h. The communication between these two brain areas might be hypothesized as a neural circuit for motivation of feeding behavior. The specificity of this coherence to hunger condition was confirmed when the coherence between these two areas was not influenced by satiety.

The present study identified the high spectral power density at delta band (2-5 Hz) in the lateral hypothalamus and theta band (8-12 Hz) in the hippocampus. These patterns are proposed to be area specific. Therefore, in hunger condition, the increases in gamma synchronization during a period of negative energy balance were seen. In general, gamma oscillation has been observed during cognitive performance tests [17, 18]. The enhancement of gamma activity was not due to hyperactive locomotor activity. In terms of mechanism, the activation of specialized GABAergic interneuron networks has been shown to produce gamma oscillations [19]. Moreover, gamma rhythm is also maintained by fast excitations through activation of glutamate receptors [20]. It was found that gamma oscillations are necessary for functional connectivity among various brain areas [21]. During hungry period, gamma oscillation was proposed to support the essential communication between the lateral hypothalamus and the hippocampus in response to hunger but not satiety. Based on various anatomical and physiological findings, pacemaker neurons that produce gamma wave oscillation in the hippocampal and neocortical networks might enhance synchronized network oscillations within the gamma band by entraining other cells with their firing patterns.

In conclusion, the data confirmed a sensitive role of the lateral hypothalamus to hunger with LFP power spectrum. The interaction of the lateral hypothalamus with the hippocampus also suggests neural mechanism that would trigger feeding related learning or behavior.

Ethical approval All procedures performed in this study involving animals were in accordance with the ethical standards of the Animals Ethical Committee of the PSU.

Acknowledgements The study was supported by the Graduated school, Department of Physiology, Faculty of Science, Prince of Songkla University, Songkhla, Thailand and the research professional development project under the science achievement scholarship of Thailand.

Conflict of interest All authors declare that they have no conflict of interest.

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