Alteration of Neural Network Connectivities Following Repeated Exposures to Palatable Food

Nifareeda Samerphob

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Physiology

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Major Program	Physiology

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I hereby certify that this work has not been accepted in substance for any degree, and is not being currently submitted in candidature for any degree.

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ชื่อวิทยานิพนธ์ การเปลี่ยนแปลงของการเชื่อมต่อวงจรประสาทหลังการได้รับอาหารอร่อยซ้ำๆ
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บทคัดย่อ

พฤติกรรมการกินอาหาร เป็นพฤติกรรมที่สำคัญของสิ่งมีชีวิต เกิดขึ้นเพื่อประโยชน์การดำรงชีวิตให้อยู่รอดและสามารถดำรงเผ่าพันธ์ไว้ได้ งานวิจัยนี้ จึงมีจุดมุ่งหมายที่จะศึกษากลไกการทำงานของระบบประสาทที่เกี่ยวข้องกับพฤติกรรมการกินอาหารในสัตว์ทดลอง โดยอาศัยการวัดศักย์ไฟฟ้ารวมเฉพาะที่ (local field potential) ภายในสมองทั้ง 4 บริเวณของหนู mice ได้แก่ lateral hypothalamus (LHa) สมองที่ทำหน้าที่เป็นศูนย์กลางในการตอบสนองต่อความหิว, nucleus accumbens (NAc) สมองที่เกี่ยวข้องกับความพึงพอใจและการเสพติด, dorsal hippocampus (HP) เป็นสมองที่เกี่ยวข้องกับกระบวนการเรียนรู้และจดจำ และ olfactory bulb (OB) สมองส่วนรับกลิ่น

ผลการศึกษาพบหนู mice แสดงพฤติกรรมชื่นชอบช็อกโกแลตเพิ่มขึ้นหลังจากได้รับช็อกโกแลตซ้ำๆ และมีรูปแบบคลื่นสมองที่เปลี่ยนแปลงไป โดยการทำงานของช่วงคลื่นเดลต้า (0.5-4 Hz) เพิ่มขึ้นในสมอง LHa, NAc และ HP และช่วงคลื่นแกมมา (30.5-100 Hz) ลดลงในทั้ง 3 บริเวณ นอกจากนี้ ความถี่เธต้า (4.5-12 Hz) และแกมมา (60-100 Hz) มีการประสานการทำงานมากขึ้นในสมอง HP และมีสัญญาณที่บ่งชี้การสื่อสารกันระหว่างสมองสองบริเวณ ซึ่งชี้ให้เห็นว่า LHa-NAc, LHa-OB, NAc-HP และ HP-OB สื่อสารกันมากขึ้นในช่วงคลื่นความถี่เบต้า (12.5-30 Hz) และแกมมา (30.5-100 Hz) หลังจากได้รับช็อกโกแลตซ้ำๆ สะท้อนการเปลี่ยนแปลงของวงจรการทำงานภายในสมองที่ถูกชักนำโดยการกินช็อกโกแลตเป็นประจำ ซึ่งอาจนำไปสู่ความชอบในการกินช็อคโกแลต นอกจากนี้ การศึกษาพบคลื่นไฟฟ้าสมองช่วงคลื่นเบต้า (12.5-30 Hz) และแกมมา (30.5-100 Hz) ทำงานเพิ่มมากขึ้นในสมองส่วน LHa, NAc และ HP ขณะอดอาหาร และการทำงานของช่วงคลื่นดังกล่าว สัมพันธ์กันมากขึ้นระหว่างสมอง LHa- HP และ NAc- OB สะท้อนให้เห็นการทำงานของวงจรประสาทที่เชื่อมต่อกันเพื่อตอบสนองต่อภาวะการลดลงของพลังงานในร่างกาย

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Abstract

Feeding is the important characteristic of living organisms being a great significance struggling for existence and survival. The aim of the present study was to investigate neurophysiological mechanisms in association to food consumption behavior in mice. Local field potential were recorded from 4 associated brain regions of feeding in mice consisting of the lateral hypothalamus (LHa) implicated to regulate food intake and motivated behaviors, the nucleus accumbens (NAc) which is a central role of reward circuit, the dorsal hippocampus (HP) or the foundation of learning and memory and the olfactory bulb (OB) or the gate of smell perception.

Animals expressed chocolate-like behavior and LFP changes following repeated chocolate consumptions. Here, delta (0.5-4 Hz) power was increased in the regions of LHa, NAc and HP, while the gamma powers (30.5-100 Hz) decreased in these brain areas. Theta (4.5-12 Hz)-high gamma (60-100 Hz) coupling was strengthened in the HP. Moreover, LHa-NAc, LHa-OB, NAc-HP and HP-OB were increased the coherence activity of beta (12.5-30 Hz) and gamma (30.5-100 Hz) oscillations following the chocolate sessions indicating the brain modulation induced by repeated chocolate learning that produce food preference to chocolate consumption. The features of brain signaling during hunger showed remarked patterns associated with food deprivation where beta (12.5-30 Hz) and gamma (30.5-100 Hz) power increased in the LHa, NAc and HP. In addition, increased beta and gamma coherence were observed in LHa-HP and NAc-OB reflecting neural circuits in response of negative energy status.

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In the name of Allah, the most gracious, the most merciful throughout my path of study, I would like to express my deepest sense of gratitude towards him from the beginning to the end of this study.

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Nifareeda Samerphob

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List of Abbreviations and Symbols

ANOVA	Analysis of varience
CFC	Cross-frequency coupling
DTFT	Discrete-time Fourier Transform
EEG	Electroencephalography
EMG	Electromusculography
FFT	Fast Fourier transform
HP	Hippocampus
ICLAS	International committee on laboratory animal science
LFP	Local field potential
LHa/LH	Lateral hypothalamus
MI	Modulation index
NAc	Nucleus accumbens
OB	Olfactory bulb
PAC	Phase-amplitude coupling
PSD	Power spectral density
S.E.M.	Standard error of mean

List of Original Publications

This thesis contains some of the research outputs published in the international refereed journals and it has been already permitted to be included in the thesis by the publishers. The following research papers are listed accordingly

Nifareeda Samerphob, Dania Cheaha, Surapong Chatpun., Ekkasit Kumarnsit. Gamma wave oscillation and synchronized neural signaling between the lateral hypothalamus and the hippocampus in response to hunger. *The Journal of Physiological Science*, 65 (Suppl 2), s-17 - s-22, 2015

Nifareeda Samerphob, Dania Cheaha, Surapong Chatpun., Ekkasit Kumarnsit. Hippocampal CA1 local field potential oscillations induced by olfactory cue of liked food. *Neurobiology of Learning and Memory*, 142, 173-181, 2017

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CHAPTER 1
INTRODUCTION

1.1 Background and Rationale

Comparing feeding habits of human in the past, eating becomes a serious problem nowadays where people increase food intake and decrease physical activity. The modern world is overwhelmed with abundance of foods, restaurants and supermarkets more than in the past, feeding habits have been devoted to the causes of much illness and deteriorated health in general. During the increased number of obese and patients in eating disorder progressively in worldwide, obesogenic environments have been hypothesized to be discussed in details of brain alteration overriding the normal physiology of eating controls leading to addiction-like behavior and obese.

The local field potential (LFP) is the electrophysiological signal that records the summation of synchronized inputs from the population of neurons around the observed area inside the brain. The LFPs display the features of firing neurons depending on the brain state. The multi-dimensional measure for studying neural network activity of feeding behavior has been made in attempt to understand at the brain mechanisms of behavior unit. Brain activity was studied in terms of LFP oscillation in correlation with homeostatic hunger and hedonic aspects which are involved with various brain sites designated to integrate for internal signals and external stimuli. Sophisticated data analysis and interpretation of signals are very essential to understand the key component of how the brains work to control eating behavioral responses.

1.2 Literature Reviews

1.2.1 Neural systems controlling eating behavior to maintain caloric balance

Foods provide source of energy for cellular processes and physical activities in living organisms. Eating behavior, therefore, is recognized to be crucial for survival of the species. Our physiological needs provide the basic determinants between the regulation of food intake (energy inflow) and energy expenditure (energy outflow) that is essential for the regulation of the central nervous system (CNS). The disturbance of the network may result in under-nutrition, over-nutrition and eating disorders (Kullmann et al., 2012). In homeostatic system, the metabolic drive is actually and basically effective for meal initiation and termination of food intake that compromises the endocrine signals arose from gastrointestinal tract and adipose tissue arose to the nervous function especially the hypothalamus and brain stem (Gahagan, 2012). The hypothalamus is the key regulator to control food intake and energy balance via the functions from many sets of neuron contained in many regions such as the arcuate nucleus (ARH), paraventricular nucleus (PVH), ventromedial nucleus (VMH), lateral hypothalamus (LHa) and perifornical area (PFA) (Simpson et al., 2009). In particular, the peripheral signals could present firstly at the two distinct neural populations in the ARH via blood stream presenting the opposite influences, orexigenic agouti-related peptide (AgRP) /neuropeptide Y (NPY) and anorexigenic pro-opiomelanocortin (POMC)/cocain and amphetamine-related transcript (CART) (Kristensen et al., 1998; Lenard and Berthoud, 2008; Mutt, 1994; Rossi et al., 1998; Stanley and Leibowitz, 1984; Williams et al., 2010). Ghrelin is well-known gut hormone that acts as the neuropeptide in the nervous system. Its stimulatory effect on food intake and energy regulation were produced mainly by stimulating NPY and AgRP neurons in the ARH (Hewson and Dickson, 2000; Nakazato et al., 2001). Adipocyte derived hormone leptin and pancreatic β cell-derived hormone insulin are concerned in the proportion of body mass of adipocytes as the feedback signal to regulate body weight, and both stimulate the POMC neurons in the ARH releasing α-melanocyte stimulating hormone (α-MSH) to suppress food intake and increase energy expenditure (Campfield et al., 1995; Chavez et al., 1995; Niswender and

Schwartz, 2003; Vergoni et al., 1986). The LHa as a major hub receives the metabolic information from first-order orexegenic peptides down-streaming widely to higher order neurons through the entire brain from the cortex to spinal cord to control food intake and wakefulness (Berthoud, 2002). Whereas, the PVH present the second-order neurons associated with the function of autonomic and endocrine outputs for energy metabolism (Lechan and Fekete, 2006). Multiple neural systems and pathways in controlling food intake are shown (Fig.1.1). In a nutshell, executive control of food intake and physical movement for voluntary control are undertaken by the cortex. Circulating signals are indicated to influence several pathways in the neural systems, while external stimuli are emphasized on interactions with learning or memory process and higher executive control of the brain cortex (Lenard and Berthoud, 2008).

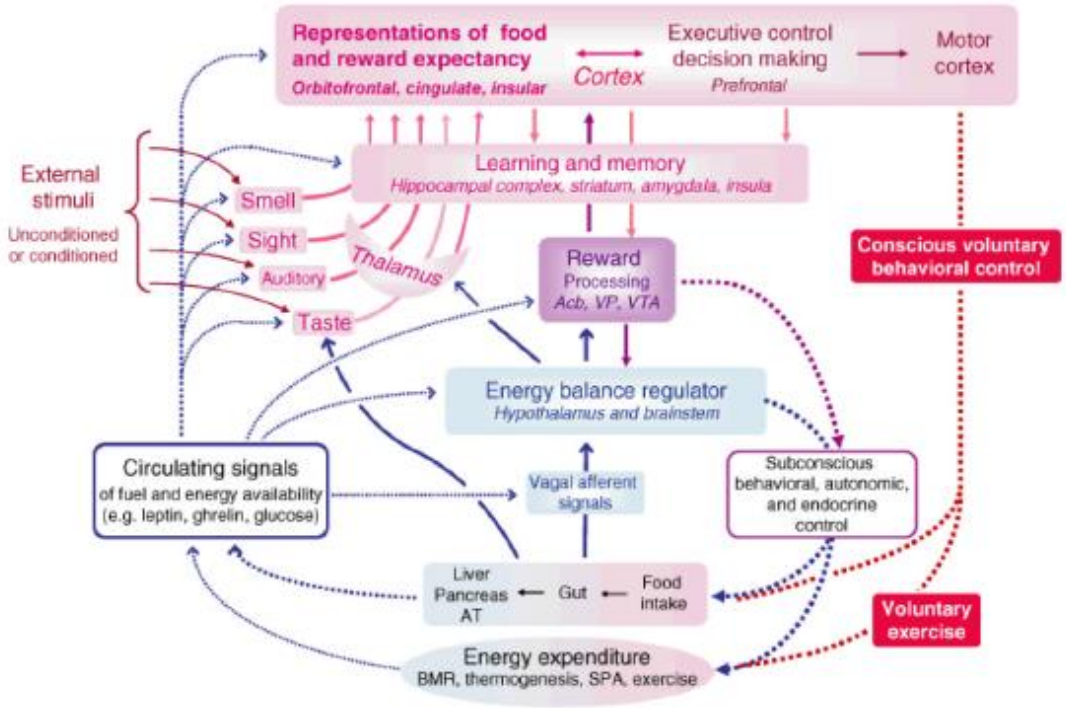

Figure 1.1 Multiple neural systems and pathways in controlling food intake (Lenard and Berthoud, 2008).

1.2.2 Non-homeostatic environmental and cognitive factors over the power of homeostatic regulation

A low point of metabolic fuels has been shown directly to induce food ingestion, even without a list of favorite food or nothing more delicious. In contrast, ingestion even in satiated state is the property of wanting and liking for palatable or high caloric foods. The realization that learning about context of food and environment enforced to work with reward system to generate the pleasurable and rewarding feelings in food consumption suggests the importance of neural network in the attempt to gain more calories dense foods (Abizaid, 2009; Rolls, 2003; Tordoff, 2002).

It is becoming more and more of interest to dissect components of food reward that are liking (emotional impact) and wanting (incentive salience). The brain opioid peptide is transmitted to the reward related areas such as the nucleus accumbens (NAc), amygdala, and ventral tegmental area (VTA) contributing to conscious pleasure or implicit liking of foods (Badiani et al., 1995; Bakshi and Kelley, 1993; Gosnell, 1988). The involvement in opiate-mediated enhancement of food intake is also linked with homeostatic regulatory brain areas such as the PVH, VMH, dorsomedial and lateral parts of hypothalamus (Gosnell et al., 1986; Leibowitz and Hor, 1982; Stanley et al., 1988). The cross-talk between homeostatic and non-homeostatic systems could yield insights with opioid responsiveness in the cortico-striato-hypothalamic circuit driven feeding via the prefrontal cortex (PFC) to the hypothalamus as “driver” pathway of feeding, and the counterbalance mechanisms of local opioids in the prefrontal cortex projecting to the NAc as “limiter” circuit transmitting to the hypothalamus (Selleck and Baldo, 2017). Although sense of pleasure especially of eating preferred foods is followed by feeling of wanting or craving to eat it repeatedly, neural substrate important in food reward or motivational process is distinct from hedonic experience factor that vastly in the mesolimbic dopamine system by increased dopamine release in the VTA to the NAc and PFC (Martel and Fantino, 1996). The NAc activation correlated with nutritionally relevant hormones, leptin and ghrelin, suggesting the reward processing in the mesolimbic dopamine system is an integral part of endogenous inhibition and orexegenic action to

influence physiological mechanisms related to feeding (Abizaid et al., 2006; Farooqi et al., 2007; Fulton et al., 2006).

The basic neurological paradigm of procurement and foraging food are learning, remembering and finding the best food source repeatedly in the same way. The hippocampus is responsible for information consolidation, retention of experiences and spatial memory allows accurate navigation (Abrahams et al., 1999; Scoville and Milner, 2000; Smith and Milner, 1981). Peripheral hormones enhanced hippocampal function in integrating internal, external, mnemonic and cognitive information to reduce (leptin, glucagon-like peptide-1) or increase (ghrelin) food consumption and learned-food reward responding (Fig 1.2) (Kanoski and Grill, 2017a). Consistently, feeding behavior is influenced by the function of the hippocampus when amnesic patients whose bilateral hippocampal damage lost new episodic memories belonging to previous meal and consumed more successive meals (Higgs et al., 2008; Rozin et al., 1998a). Moreover, the explicit recall of previous meals aids to reduce the amount of food to consume (Higgs, 2002). Rather, hippocampal neural processing is required for food-related cues associated with rewarding effect of post-ingestive nutrition consequences that influence to subsequent meals (Benoit et al., 2010; Davidson et al., 2007). Collectively, episodic meal-related mnemonic information and condition-associative learning on feeding has primed to regulate the amount of next meals and hunger levels.

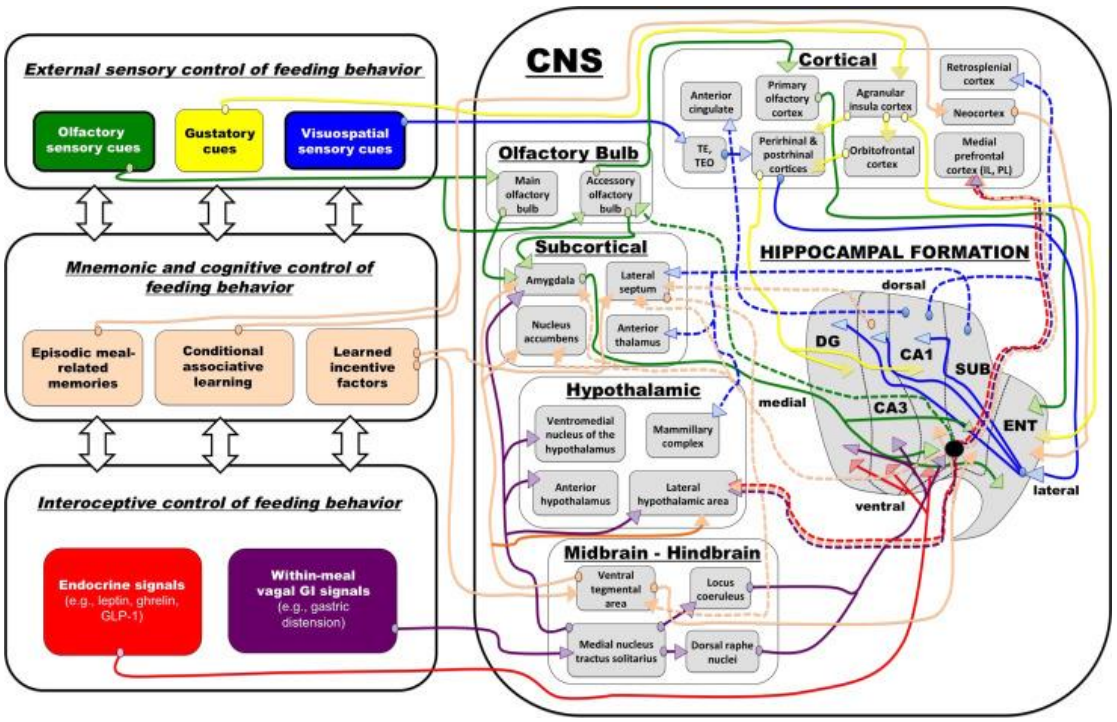

Figure 1.2 Neuroanatomical interconnectivity in associated feeding regions. Hippocampal formation for the integration of external sensory food-relevant information and internal contextual cues has been focused (Kanoski and Grill, 2017b).

1.2.3 Local field potentials (LFPs) and neural processing

Extracellular voltage fluctuations (local field potentials**,** LFPs) represent neural mass action prevailing in accordance of brain sites and species. Several studies elucidate the characteristics of LFP signals underlying in association of spiking activity and synchronous event of small populations of neurons correlated with several brain states (Arieli et al., 1996; Berens et al., 2008a; Henze et al., 2000; Logothetis, 2003). Using extracellular electrodes embedded directly in the brain region, the mean field potential of electrical activity generated from various neural process events around the tip of electrode is recorded (Fig 1.3). The high frequency bands between 0.6-3 kHz are referred to the events of spikes activity from single or multiunit activity over the distance of 140-300 μm (Gray et al., 1995). On the other hands, low frequency extracellular voltage fluctuations lowering than 200 Hz are considered to aspects of neural ensemble and dendritic processing as synaptic synchronization, sub-threshold membrane potentials and potentials following firing

activities (Mitzdorf, 1985). More details of LFP are identifiable components of human cognitive system included sensory perception, memory and attention (Fries et al., 2001; Gail et al., 2004; Pesaran et al., 2002; Taylor et al., 2005; Wilke et al., 2006). In addition, the sustained oscillations of LFP allow efficiently for decoding brain activity in brain machined interface to control of movement (Andersen et al., 2004; Mehring et al., 2003). Synchronized neural oscillations are subdivided into bandwidths conventionally composted of delta (1-4 Hz), theta (4-8 Hz), alpha (8-13 Hz), beta (13-30 Hz) and gamma (30-150 Hz) (Buzsaki and Draguhn, 2004). Among these rhythms, brain regions have specific features of neural unit activity and show oscillatory behaviors to be specific frequency linked to brain processing, stimulus or task related. Likewise, theta frequency oscillation is particularly apparent in hippocampal area CA1(Gillies et al., 2002), and LFPs of hippocampal CA1-CA3 pathway provide underlying indication of theta and gamma rhymes modulated during perception and memory task (Xu et al., 2012). Furthermore, gamma activity increase was present in the primary visual cortex during visual stimulation (Berens et al., 2008b), and theta rhythm in the thalamus to medial prefrontal cortex was lower in chronic stress rats, and recovered after the treatment of anti-depression drug (Zhang et al., 2011). With aspects of LFPs, the dynamics of LFP could indicate the activity of neural population typically for the use in neurophysiological studies and clinically useful.

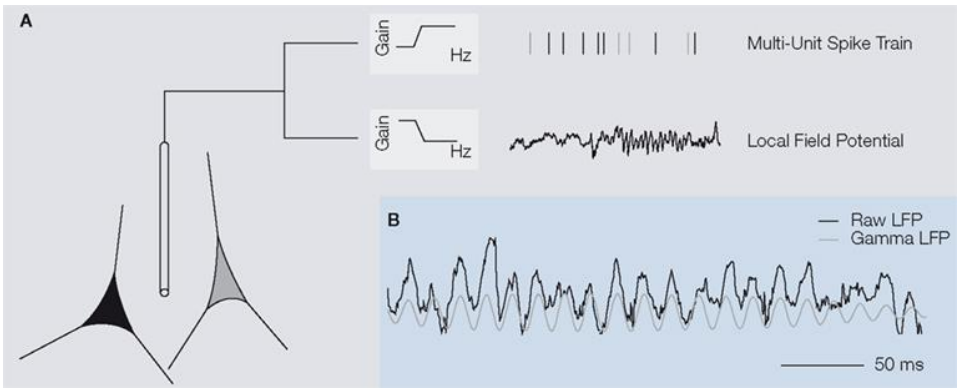

Figure 1.3 The extracellular field potentials recording in the brain. High frequency ranges of multi-unit spiking activity (top) and low frequency ranges of individual action potentials summation (bottom) can be carried out (A). Raw LFP signals in primary visual cortex of awake primate and filtered gamma frequency band (30-90 Hz) dominantly during the present of visual task (B) (Berens et al., 2008a).

1.2.3.1 Basic concept of spectral estimation

The LFPs contain such dynamic flow of information across neural network. Through the properties of the digital signal, the natural traits of the LFP tracing reveal a number of discrete points that appear as a continuous curve observed by visual inspection (Fig 1.4A-B). The space during the points is dependent on sampling frequency setting. There are many techniques to study the periodic behaviors of the signal. The power spectrum is the quantities method to indicate the amplitude of rhythmic activity in the signal in frequency domain. The dominant frequency can be observed by the apparent peak (Fig 1.4 C).

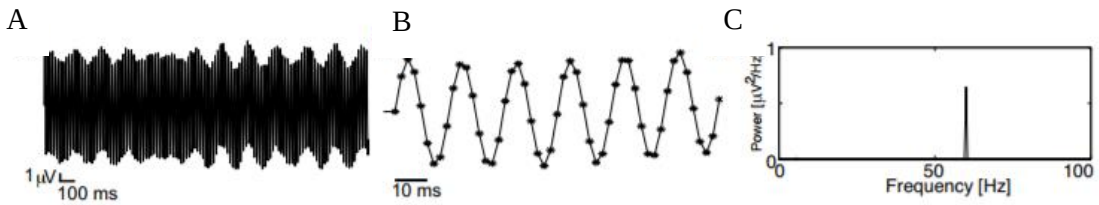

Figure 1.4 A digital signal analysis in frequency domain. 100 ms simulated signals depicted the content of analog values converted to digital value (A, B). Algorithm is conducted to transform signals in time domain to frequency domain (Kramer, 2013).

Throughout the version of field analysis, the power spectrum in the general area of signal processing based on the assumption of data conducting to two mathematical methods. The data assumed to be in the normal distribution is efficiently model for the parametric method to display the stationary stochastic process, while the regardless of data population in case of random signals is considered to use nonparametric method (Stoica and Moses, 2005). The classical nonparametric methods are sensitive as an alternative backup when parametric approaches are not met (Maris and Oostenveld, 2007; Oostenveld et al., 2011). In the interest of the signals in the real world, the main motivation for focusing on complex characteristics of ergodic process with varying amplitudes and frequencies is said to the nonparametric spectral estimation that let us to consider the rhythmic activities contained in the signals $x(t)$ in time series at different frequency components. An example of steps of spectral analysis reveals the splitting the signal into samples. Thereafter, each segment is multiplied by the window function resulting in the windowed signal. Likewise, the discrete time-Fourier transform (DTFT) displays

frequency spectrum of the signal. The last step is the computing an average of squared magnitude of frequency spectrum to reduce the random noise (Smith, 1997). The construction of power spectra is basically applied by the mathematical analysis; Fourier transform such as discrete Fourier transform (DFT) (Kay and Marple, 1981). The function of Fast Fourier transform (FFT) is efficient algorithm in implementation of DFT. What the Fourier transform do with the signal $x(t)$ waveform in a function of time is, in the example (Fig 1.6), the action of rotating the waveform $x(t)$ in the clockwise direction in horizontal plane allowing the side view of the wave. The power spectrum of the signal is often plotted in the relation of square amplitude (known as power) in frequency domain representation. Because a random signal actually is more easily to encounter the average power than infinite energy, the power spectral density (PSD) is defined for that quantity of continuous signal under stationary process over all time. The density means the power estimation in small frequency ranges or bins. Stoica and Moses explain the spectral calculation simply through simple equation at the beginning of the determination of discrete time data sequences (Stoica and Moses, 2005). For total energy of the signal in finite time interval, mathematical modeling of energy for the continuous time signal $y(t)$ is expressed as following;

$$\mathrm{E} = \sum_{t=-\infty}^{\infty} |y(t)|^2 \tag{1.1}$$

Then the energy spectral density per unit frequency contained in the signal at frequency f is shown following this equation;

$$S(\omega) = |Y(\omega)|^2 \tag{1.2}$$

The right solution is the Fourier transform of energy content contained in the signal, and the square of the Fourier transform of the signals energy is called as the density function. The discrete time-Fourier transform (DTFT) of the signal is expressed as this;

$$Y(\omega) = \sum_{-\infty}^{\infty} y(t) e^{-iwt} \tag{1.3}$$

In common, the power spectral density is concentrated more for continuous signal over all time due to an infinite energy. Here, the PSD of random signal in time series X_n can be expressed in one of two ways that are equivalent to each other. The spectral

density $\emptyset(\omega)$ determining on the DFTF of the covariance sequence is derived by sampling a continuous time-signal at infinite length as (1.4).

$$\emptyset(\omega) = \lim_{N\to\infty} E\left\{\frac{1}{N}\left|\sum_{t=1}^{N} y(t)e^{-iwt}\right|^2\right\} \quad (1.4)$$

When ω refers to number of radiances per sampling interval and can be subset values from$-\pi\ to\ \pi$, and E is the expectation operator that averages all variable factors. Note that there is one for PSD calculation, the signals are seen as Fourier transform pairs among the PSD and autocorrelation function.

$$\emptyset(\omega) = \sum_{k=-\infty}^{\infty} r(k)e^{-i\omega k} \quad (1.5)$$

Which $r(k)$ is the autocorrelation function denoting to inverse transform of given $\emptyset(\omega)$

$$r(k) = \frac{1}{2\pi}\int_{-\pi}^{\pi} \emptyset(\omega)e^{i\omega k}d\omega \quad (1.6)$$

The periodogram is the approach for PSD estimation underlying the process of Fourier transform and square-magnitude component of DFT.

$$P_{xx}(f) = \frac{1}{LF_s}\left|\sum_{n=0}^{L-1} X_L(n)e^{-j2\pi fn/Fs}\right|^2 \quad (1.7)$$

As the number of samples used in the computation increase, Welch's method is alternative to reduce the variability of the periodogram breaks the time series into overlapping segments by averaging number of samples over times (Fig 1.7).

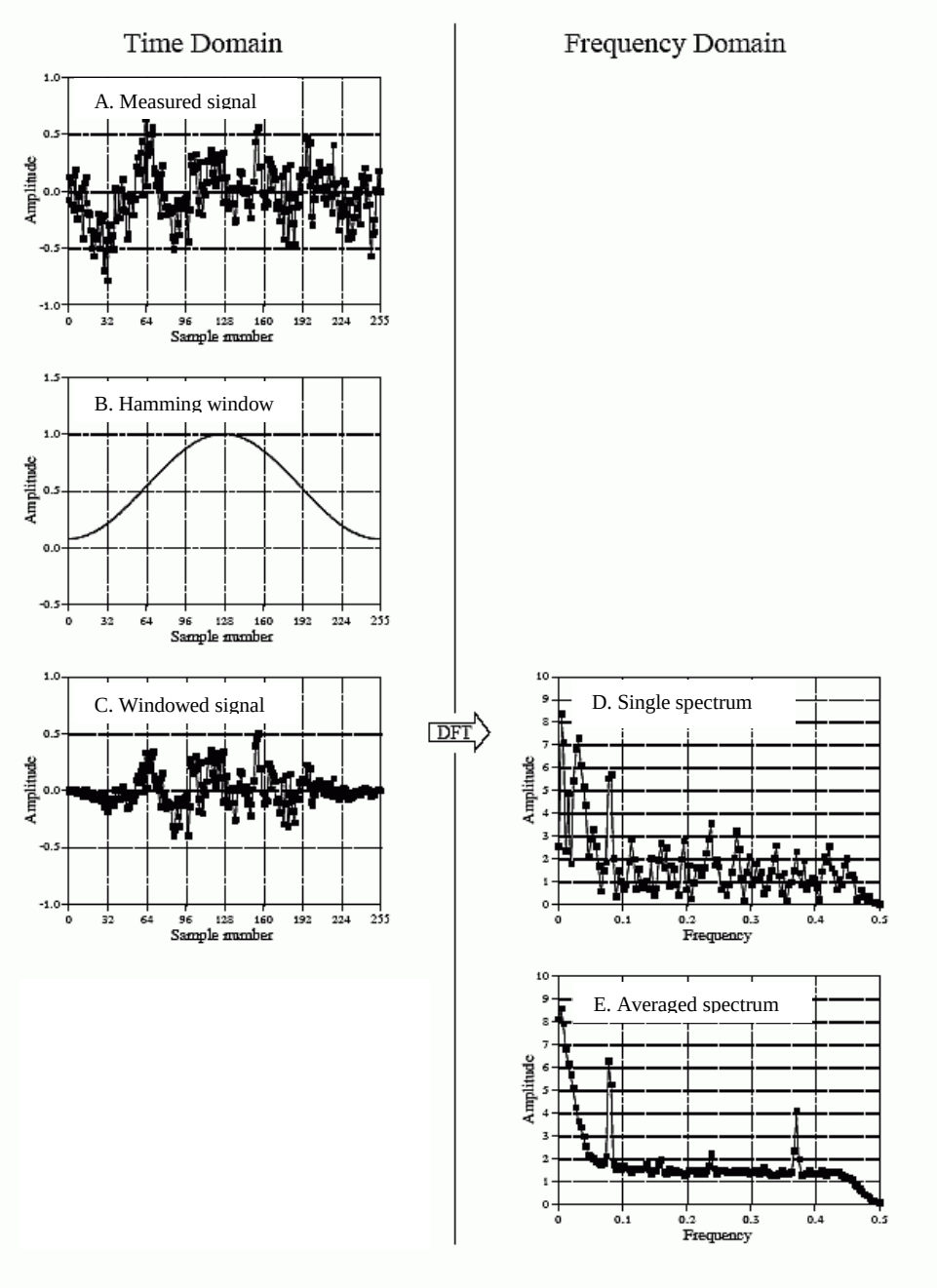

Figure 1.5 Multi-steps of spectral analysis for measured signals. Measured signal (A) was applied by window function (B) along short segments of the signal to windowed signal (C). Each segment of windowed signal was performed by algorithm to calculate single spectrum (D), and finally they were averaged for frequency spectrum (D) (Smith, 1997).

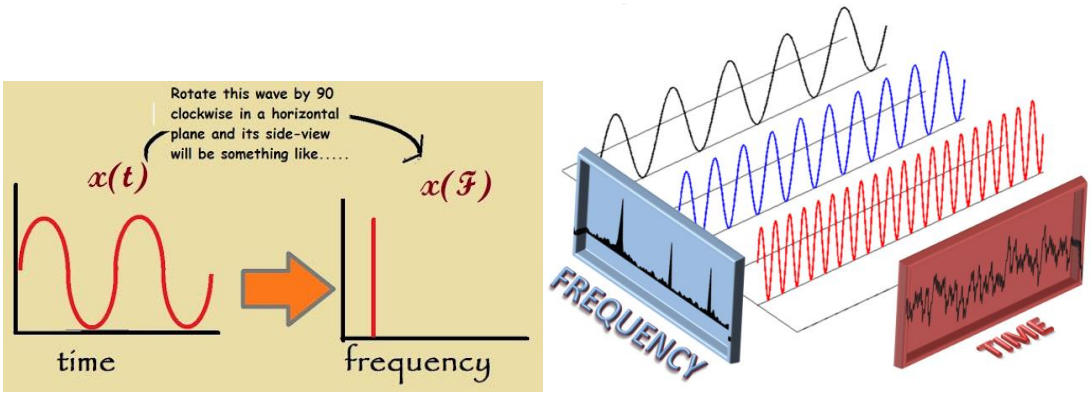

Figure 1.6 Fourier transforms algorithm rotate the signal by 90 degree clockwise in the horizontal plane resulting in its side-view observed in frequency domain (http://visualizingmathsandphysics.blogspot.com).

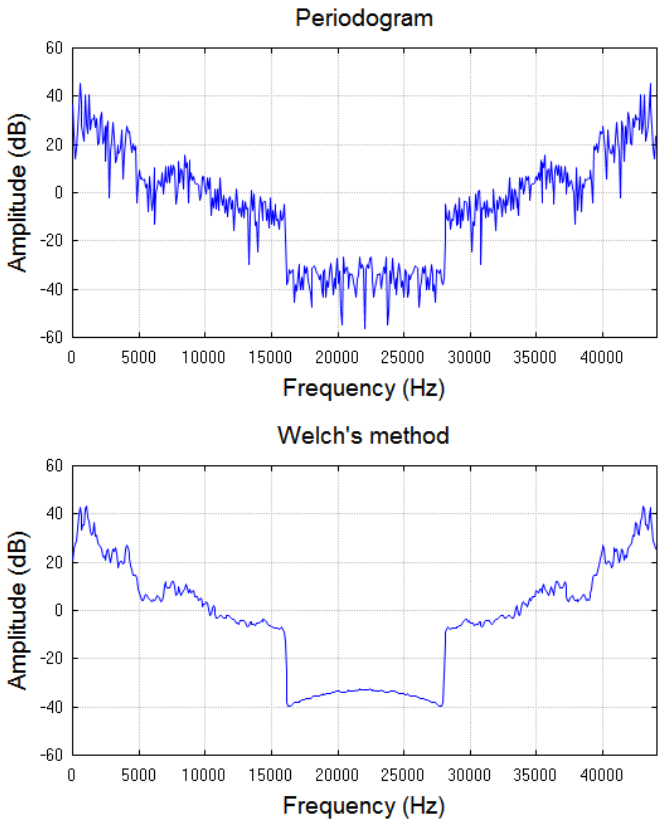

Figure 1.7 Power spectral density estimation for signals by periodogram and Welch methods (averaging periodogram) (https://en.wikipedia.org).

1.2.3.2 Inferring a functional connectivity: spectral coherence (SC)

From the fact that the brain-circuits are taken into consideration of different brain regions connected with each other to work and process the information, the spectral coherence (SC) is a normalized measure of the interaction between regions through the cross-spectrum given by $P_{xy}(\omega) = P_x(\omega)P_y^*(\omega)$ of two signals, $x(t)$ and $y(t)$ recorded at different sites

$$SC_{xy}(\omega) = \frac{P_{xy}(\omega)}{\sqrt{P_{xx}(\omega)P_{yy}(\omega)}} \tag{1.8}$$

The $P_{xx}(\omega)\ and\ P_{yy}(\omega)$ are the autospectral density of x and y respectively. The range covers 0 to 1 in which the yield is equal to zero, this mean, and then there is no interaction between two measured signals. In case of SC result is equal to 1, it is indicated a perfect linear relationship among the processes or the explaining the positive of completely functional connectivity (Castellanos and Makarov, 2006). Indeed, the coherence suggests the phase consistency between signals. Therefore, we could say that the strong coherence is from the constant phase relationship, and the weak coherence reflect the random phase relationship over trials between the two signals at frequency index (Fig 1.8). An example of coherence spectrum showed the high value at frequency 24 Hz and weak at other frequencies (Fig 1.9).

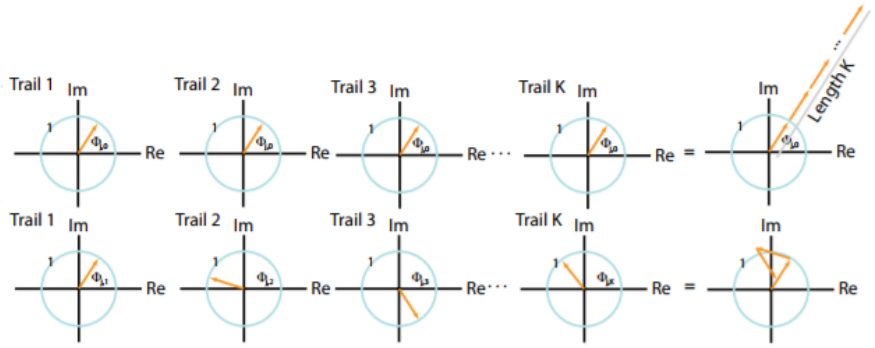

Figure 1.8 The phase difference in a complex plane over trials. Upper case represents phase for the same direction along trial K producing a long resulting vector shown in the last column. Lower case represents the phase difference from 0 to 2π for each trial producing the resulting vector near the origin (Kramer, 2013).

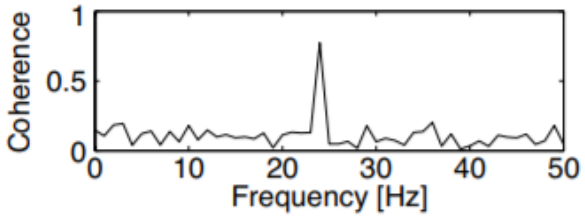

Figure 1.9 The strong coherence at frequency 24 Hz between signal x and y (Kramer, 2013).

1.2.3.3 Rational for measuring phase-amplitude coupling between neuronal oscillations of different frequencies

There has been much of particular interest to electrophysiological brain oscillations and the interaction between distinct frequency bands. In the way that different frequency oscillations show the dynamic coupling patterns, cross frequency coupling (CFC) is the common method to analyze the interaction of rhythms in continuous electrophysiological signals (Jensen and Colgin, 2007). Phase-amplitude coupling (PAC) or nesting is known to meet the interaction between different frequency oscillations in the form that the amplitude of high-frequency is modulated by the phase of low frequency (Seymour et al., 2017). For decades, this type of coupling has been linked with relation of neural mechanisms during several behavioral tasks, in particular, the cognitive aspect and sensory processing (Buzsaki and Draguhn, 2004; Demiralp et al., 2007; Schroeder and Lakatos, 2009).

The modulation index (MI) is able to track the intensity of coupling, and can be seen from the amplitude distributions (Fig 1.10). The measures of PAC are reviewed for 7 methods; the heights ratio, the power spectral density of amplitude envelop, the mean vector length, the phase-locking value, the correlation coefficient, general linear model and the coherence value. In this section, we quickly reviewed only the ways to find MI through mean vector length and coherence value. To measure MI by mean vector length, each instantaneous fast oscillation amplitude component in time is represented by the length of complex vector, whereas the slow oscillation phase of the same time point is represented by the vector angle (Tort et al., 2010). The general procedures are included the filtering of two interested frequency ranges, extraction of phase for low frequency (f_p) and amplitude for high frequency

(f_A) before the construction of phase-amplitude distribution plot which gives the amplitude of f_a oscillation at each phase of f_p rhythm is performed (Fig 1.11). Therefore, the MI by mean vector length method is calculated vector length of z (t) in the complex plan, when z (t) is defined to $A_{fA}.e^{i\emptyset f_p}$. Additionally, A_{fA} is the envelop of fast oscillation and $\emptyset f_p$ is the phase of slow oscillation. The lack of coupling can be observed by a small mean vector length, and having no doubt that the existence of the coupling leads to larger vector.

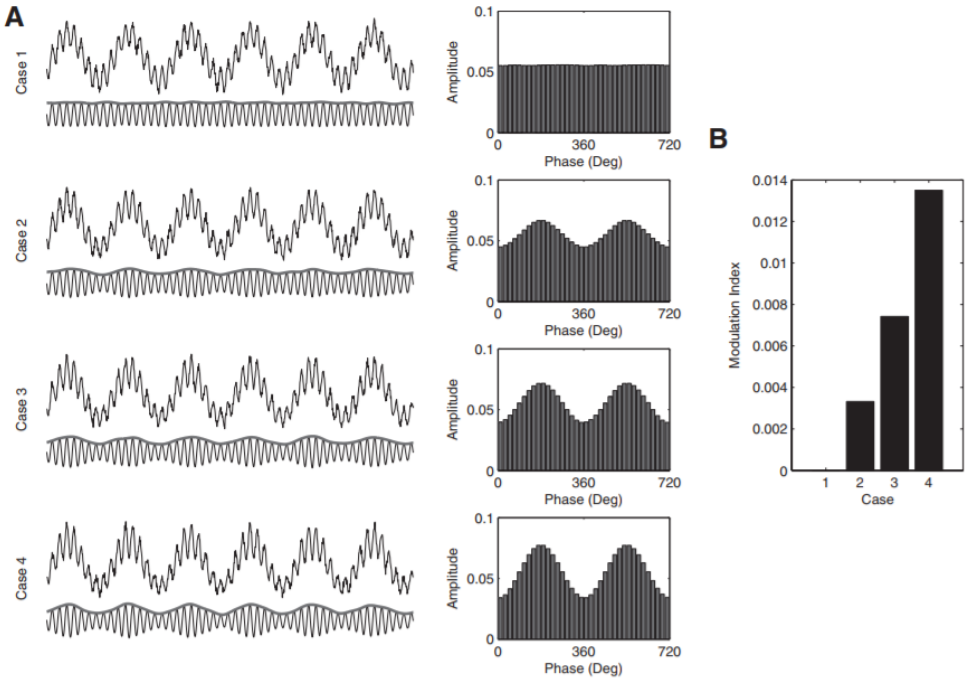

Figure 1.10 Case differing in coupling strength. Tracings between slow frequency oscillation and fast frequency oscillation and it amplitude envelop, and phase-amplitude coupling plots in four cases are shown (A). Modulation indexes for coupling were plotted according to the cases (B) (Tort et al., 2010).

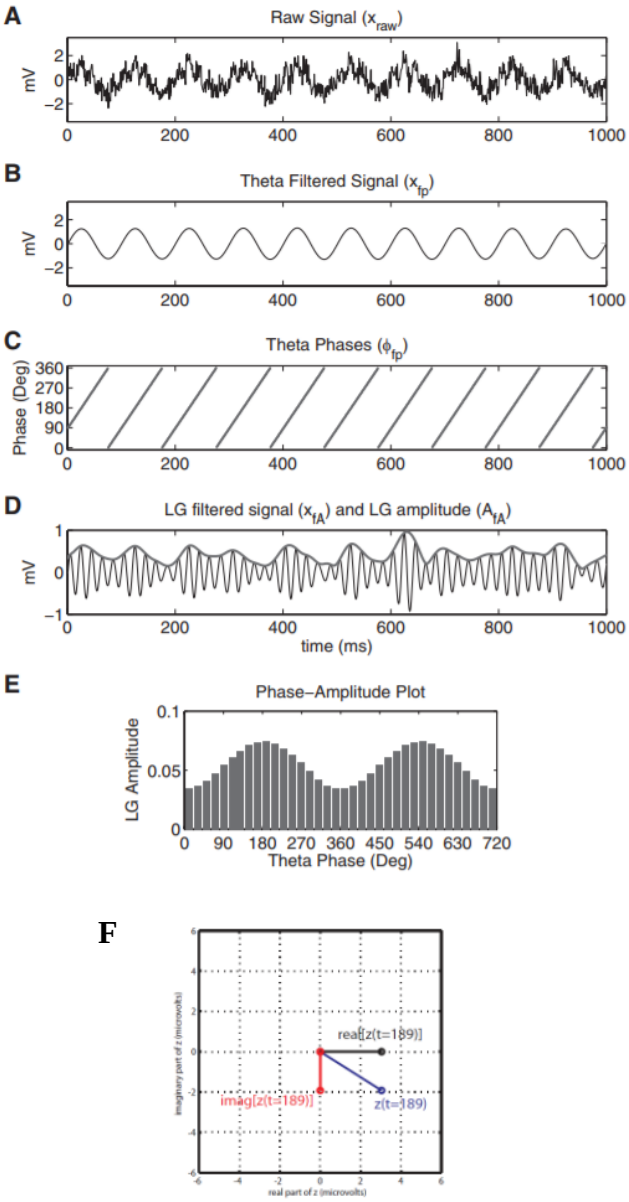

Figure 1.11 The computation of PAC of the simulated signal. Raw signal in time domain (A) was filtered for theta range (B) and calculated their phases (C). Gamma filtered signal and amplitude estimation were also obtained (D). Phase of theta and amplitude of gamma in time series were obtained to build the amplitude distribution over phase bins (E). The coupling index was evaluated from the length of vector in the complex plane for the real and imaginary parts of data (F) (Tort et al., 2010).

More recently, there is a different tool for PAC assessment based on the absolute value of coherency inferring the interaction between slower frequency signal and the time-series of the power at higher frequency (Osipova et al., 2008). Estimation of power of the signal at higher frequency (y^v), the time-course of power signal x_N was applied by sliding tapered time window and discrete Fourier transform (Fig 1.12). Next, using a standard Fast Fourier Transform (FFT) is to estimate cross spectral densities of segmented data following applying a Hanning window (h).

$$X^s = FFT\ (h^T x^s, n_{FFT})$$

$$Y^{v,s} = FFT(h^T y^{v,s}, n_{FFT})$$

The $y^{v,s}$ is the power of higher frequency at frequency v and n_{FFT} is defined to the number of FFT and frequency resolution. The term of cross-frequency coherence is calculated from the cross spectra (ξ) from individual segments defined as $\xi = X^s(Y^{v,s})^*$ and $*$ is for complex conjugate. The equation is denoted as followed;

$$\eta(v,f) = \frac{|\sum_{s=1}^{S} \xi^{v,s}|}{\sqrt{\sum_{s=1}^{S}|X^s|^2 \cdot \sum_{s=1}^{S}|Y^{v,s}|^2}}$$

For more details, the general framework for CFC estimate is described (Jiang et al., 2015).

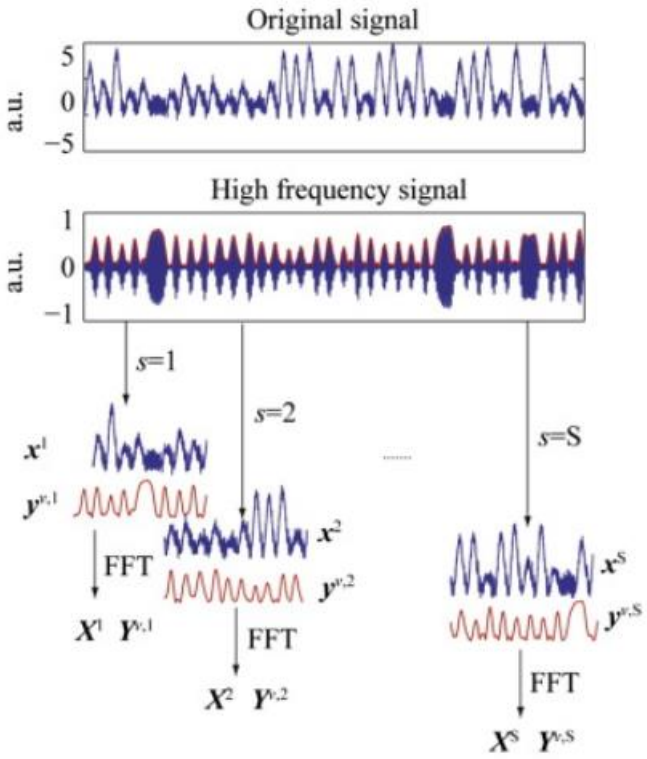

Figure 1.12 Steps of CFC estimation by coherence method. Sliding Hanning tapered window and Fourier transform represented in red line are applied to original signal to compute high frequency power at frequency v. The segments of both original signal and power signal at frequency v are transformed by Fourier analysis, thereafter cross-spectra between them are computed (Jiang et al., 2015).

1.3 Research Objectives

The purpose of this study is to investigate functional interaction among feeding-associated brains (the lateral hypothalamus (LHa), nucleus accumbens (NAc), olfactory bulb (OB) and dorsal hippocampus (HP)) emphasizing on energy reinforced feeding and motivation to food preference. To understand this central regulation to food intake, local field potential (LFP) recording within the regions was measured in correlation with energy levels and repeated chocolate consumption in satiated mice. With this experimental design, it is possible to investigate how the brains process enabled behavioral patterns of feeding.

The following objectives can be drawn;

1.3.1 To investigate the synchronized neural signaling responsible for homeostatic hunger

1.3.2 To investigate the neural network activity implemented chocolate-liked behavior

CHAPTER 2
RESEARCH METHODOLOGY

2.1 Materials

2.1.1 Electrode implantation

2.1.1.1 Surgical tools

2.1.1.2 Stereotaxic instruments (Narishige Scientific Instrument Lab., Setagaya-Ku, Tokyo, Japan)

2.1.1.3 Saeyang marathon-3 SDE-H37L1 high rotary micro motor hand piece electric tools

2.1.1.4 Tungsten carbide burs (Handpiece&RA)-HM1SQL

2.1.1.5 Tungsten carbide burs (Handpiece&RA)-HM33

2.1.1.6 Stainless optician screws

2.1.1.7 PFA-coated silver wire (A-M system, Sequim, Washington, United States) diameter 0.0190 inch

2.1.1.8 Balance (Model CC023D10ADBAAA, Avery Barkel, United Kingdom)

2.1.2 Chemical agents and drugs for intracranial surgery

2.1.2.1 Atropine sulphate 1/100 GR (A.N.B. Laboratories Co., Ltd., Thailand)

2.1.2.2 Lidocaine hydrochloride (Locana, L.B.S. Laboratory Ltd., Part., Thailand)

2.1.2.3 Ketamine (Calypsol, Gedeon Richter Ltd., Hungary)

2.1.2.4 Xylavet (Xylavet, Thai Meiji Pharmaceutical Co., Ltd., Thailand)

2.1.2.5 Vidisic eye gel (Bausch&Lomb/Dr.Mann Pharma, Germany)

2.1.2.6 Acrylic resin (Unifast Trad, GC Dental Products Corp., Japan)

2.1.2.7 Ampicillin (General Drugs House Co., Ltd., Thailand)

2.1.2.8 Thiopental sodium (Jagsonpal Pharmaceuticals Ltd., India)

2.1.2.9 Alcohol 70%

2.1.2.10 Normal saline solution

2.1.2.11 Betadine solution antiseptic

2.1.2.12 Dettol antiseptic liquid

2.1.3 Intracranial and behavioral recording

2.1.3.1 Computer

2.1.3.2 Bio Amp (AD Instrument Pty Ltd, Sydney, Australia)

2.1.3.3 PowerLab (AD Instrument Pty Ltd, Sydney, Australia)

2.1.3.4 Video camera

2.1.3.5 Cylindrical apparatus

2.1.3.6 Place preference liked apparatus

2.2 Animal Subjects

Male Swiss Albino mice were obtained from the Southern laboratory animal facility, Prince of Songkla University, Thailand. The body weight during start of the experiment was about 35-45 g. Animals were housed in sterilized individual cages (26 cm × 33 cm × 15 cm) with saw dust bedding and ad libitum access to food and water. The room was constantly at 22 ± 1 °C room temperature and 50-60% humidity, and light was set up to 12/12 h cycle. Complete and balanced dietary pellet (Smart heart, Hamster food completed & balanced formula) contained proper level of nutrients briefly consisting of ~24% protein, ~4.5% lipid, ~5% fiber, ~10% humidity, ~10%

ash, ~1% calcium and ~0.7% phosphorus including additional vitamin A, D3 and E which are suitable for rats, mice and other related species. All animal care and use in this study were carefully performed according to the guidelines of the European Science Foundation (Use of Animals in Research, 2001) and International Committee on Laboratory Animal Science, ICLAS (2004). Compliance with ethical standard was approved by the animal ethical committee of Prince of Songkla University (approval ID MOE 0521.11/842).

2.3 Intracranial Electrode Implantation

Atropine sulphate injection intramuscularly for 15 minutes before surgery was undertaken to animals to prevent secretion during execution. After deep anesthesia with a cocktail of ketamine and xylaxine at the dose of 150 mg/kg and 15 mg/kg respectively via intramuscular injection, animals were placed and fixed to right position by stereotaxic holder. To maintain animals' body temperature during surgery, position lamp beside the surgical apparatus was recommended to keep animals in a warm. After shaving hair off, cleaning with alcohol and applying betadine antiseptic solution to the skin covering the head, subcutaneous lidocaine was applied to the dorsal scalp for analgesic effect from midline sagittal skin incision.

After removal of dust of dura and blood by sweeping and making the scalp dried, burr holes (approximately 1 mm in diameter) were drilled coordinating to the left sides at the position of olfactory bulb (AP: +4.5 mm, ML: 1 mm, DV: 2 mm), nucleus accumbens (AP: +0.8 mm, ML: 1 mm, DV: 4.8 mm), lateral hypothalamus (AP: -1.5 mm, ML: 1 mm, DV: 5 mm) and dorsal hippocampus (AP: -2.5 mm, ML: 1.5 mm, DV: 1.5 mm). Reference electrode was rightly in a specific position of the cerebellum (AP: +6.5 mm, midline, DV: 2 mm), and 4 additional holes were anchored screws (Fig 2.2). This was done with the use of mouse brain atlas (Paxinos and Franklin, 2004), and the diagrams in observed regions are displayed (Fig 2.4A-D). Upright of electrodes were delicately gathered into a plastic six-pin connector (Fig 2.1), while the tips were 1 mm uncoated and placed to the specific positions of the regions. Four additional screws were attached to the skull to ensure proper anchors, and adhesive glue was used to tight electrodes including screws over the scalp before full coverage with dental acrylic (Fig 2.3). Thereafter, povidone-iodine was used for wound cleansing and debridement. To prevent infection from surgery procedures,

antibiotic ampicillin (100 mg/kg) was given once a day for 4 consecutive days and allowed to fully recovery with intensive cares (body weight and intake assessments for a week).

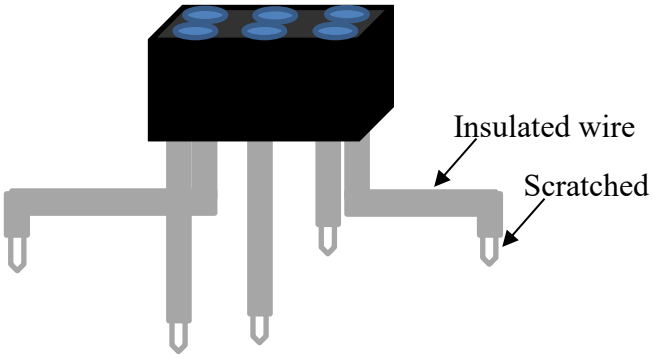

Figure 2.1 Schematic electrode construction

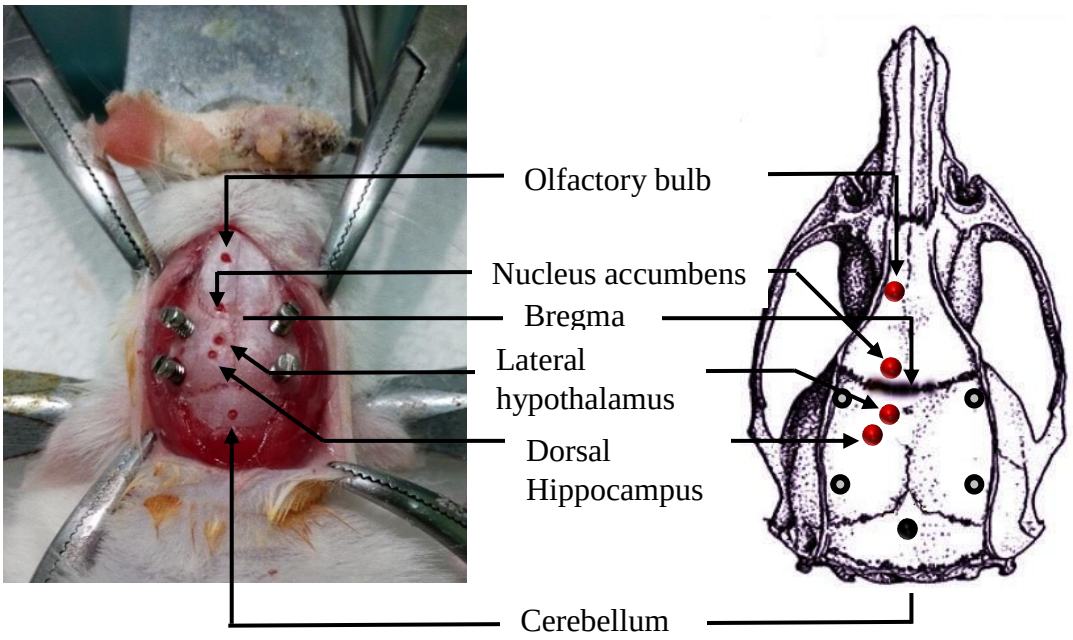

Figure 2.2 Intracranial electrode implantation in mice**.** Dorsal views depict specific areas of the left side of regions on the skull identified by using stereotaxic instrument coordinating to mouse brain atlas.

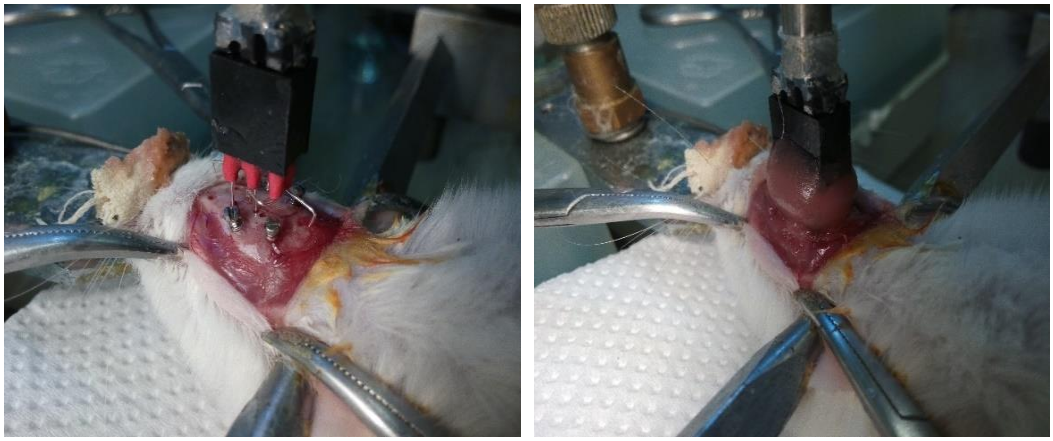

Figure 2.3 Stereotaxic placement of electrodes and dental acrylic fixation

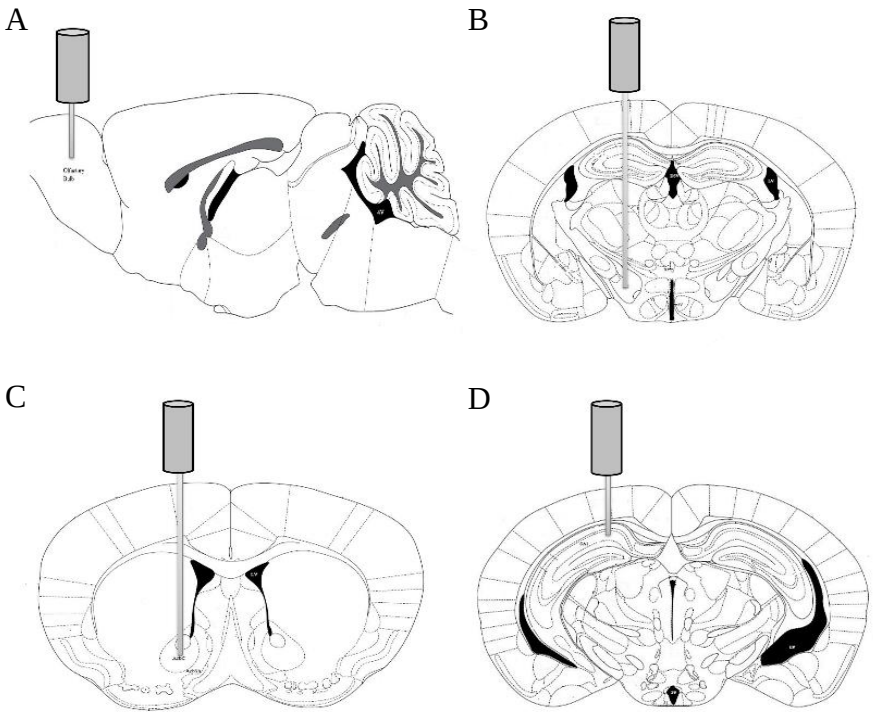

Figure 2.4 Diagrams of mouse brain section according to the mouse brain atlas (Paxinos and Franklin, 2004) located to the olfactory bulb (A), lateral hypothalamus (B), nucleus accumbens (C) and dorsal hippocampus (D).

2.4 Electrophysiological Recording, Acquisition and Data Analysis

Neural activity from ongoing intracranial potential recording of electrodes implanted in the regions of LHa, HP, NAc and OB in freely moving mice were obtained and taken previously to preamplifier (Bio Amp, AD Instrument). The PowerLab (16/35 AD Instrument, Australia) had multiplication the output digitized to a 16-bit by ADC (analog to digital converter). The sampling process was handled through a sampling of 2 kHz and 1-200 Hz band-pass filter, and notch filtering at 50 Hz was activated. The LFP digital signals were off-line recorded to personal computer by using LabChart 7.3.7 Pro software (AD Instruments, Australia). During experimental observation, animals were obtained in the experimental apparatus where the connector on their head was connected to the input connectors of the set of recording (Fig 2.5).

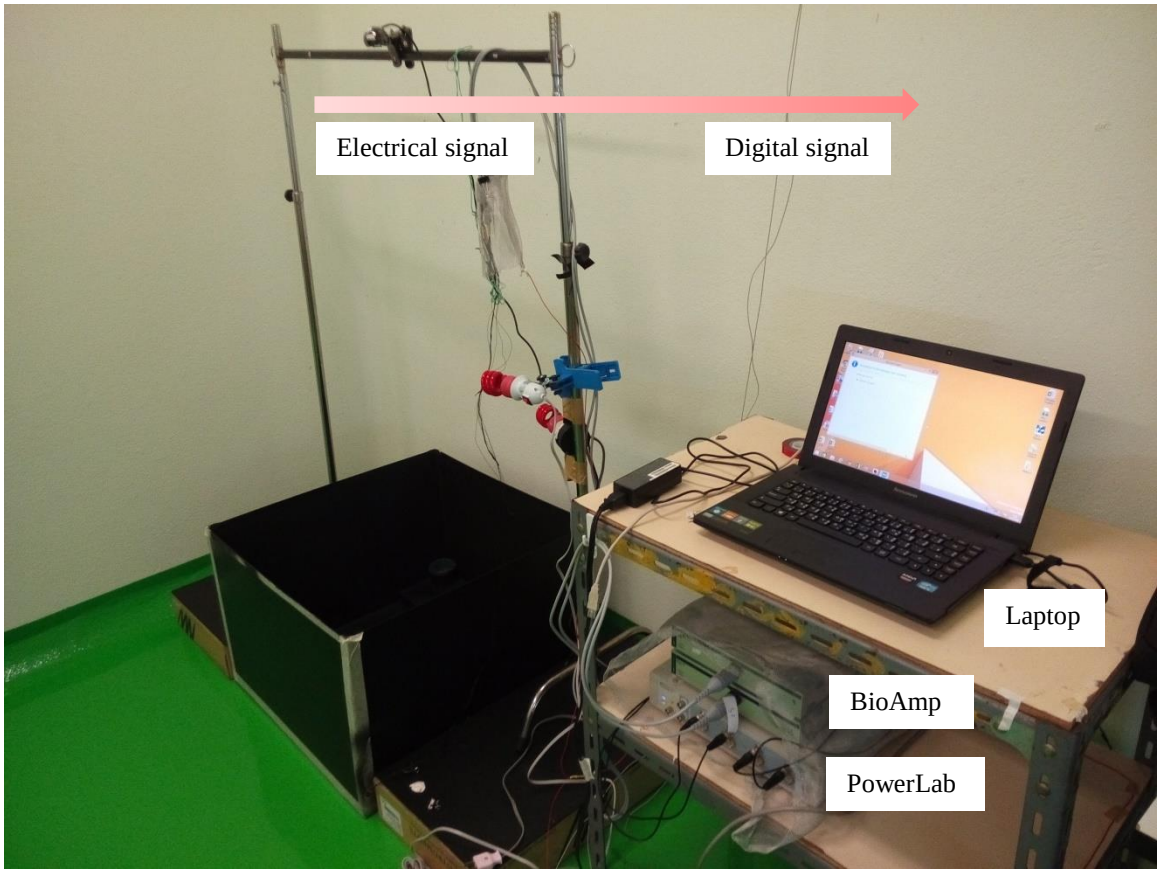

Figure 2.5 Data acquisition during experimental observation

2.4.1 Power spectral density analysis

The frequency analysis results in this study were run proficiently by the LabChart software including Brain storm toolbox. LabChart is quick for real-time signal features analysis as multiple channels are depicted as color codes in spectrogram of spectrum view against time and frequency. The power spectral density (PSD) plot and spectrogram allowed exploring according to the time epochs of interest by the setting of calculation at 2k Hz FFT size and default Hanning window (cosine-bell) with 50% window overlapping. The averaged PSD from animals in groups of condition were determined (Fig 2.7). Some common analysis can determine in LabChart is that data pad calculation brought up to a number of spectral parameters in real-time such as maximum power, maximal power frequency or mean power frequency of a selection data. To enhance visual modality, the scale of power spectrum can be adapted to logarithmic values.

The similar parameter setting was obtained for PSD estimation using the routines available in Brain storm toolbox. The off-line data extracted from LabChart were imported and pre-processed of band-pass and notch filtering for all sensor types in the toolbox. The analysis were based on the Welch method in which short segments were introduced to the Hamming window function with 50% overlapping before Fourier transform estimation and the average values in all overlapping windows were evaluated automatically (Fig 2.8). Spectral power in the sets of observations were insight for frequency ranges of the general frequency bands that included delta, theta, alpha, low beta, high beta, low gamma and high gamma (Fig 2.6).

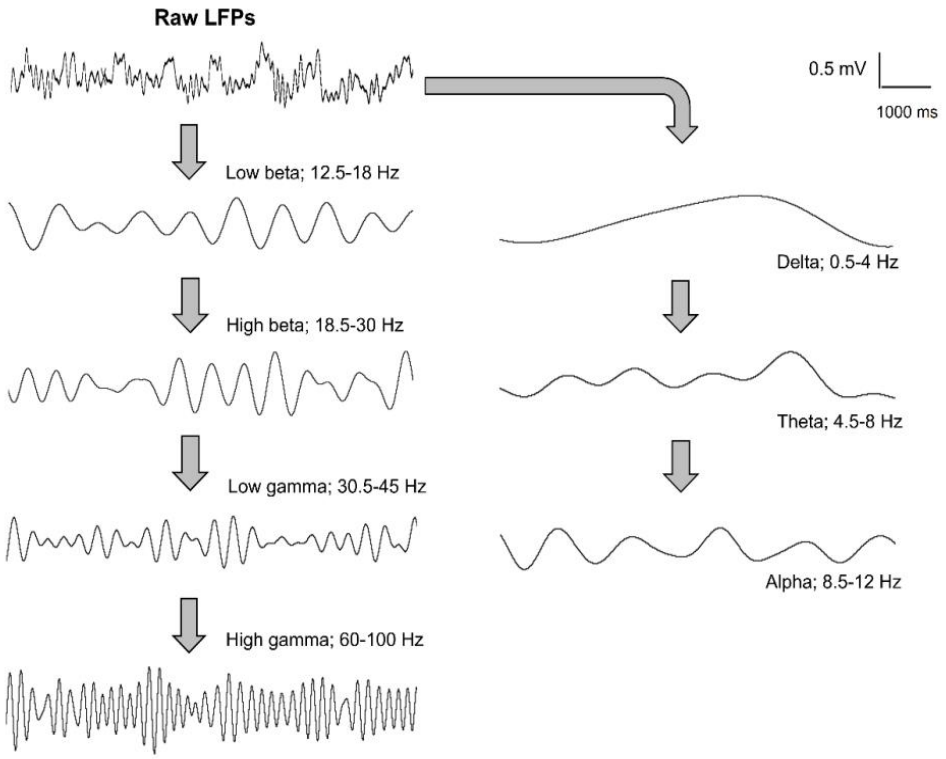

Figure 2.6 Frequency oscillation types of mouse LFP wave

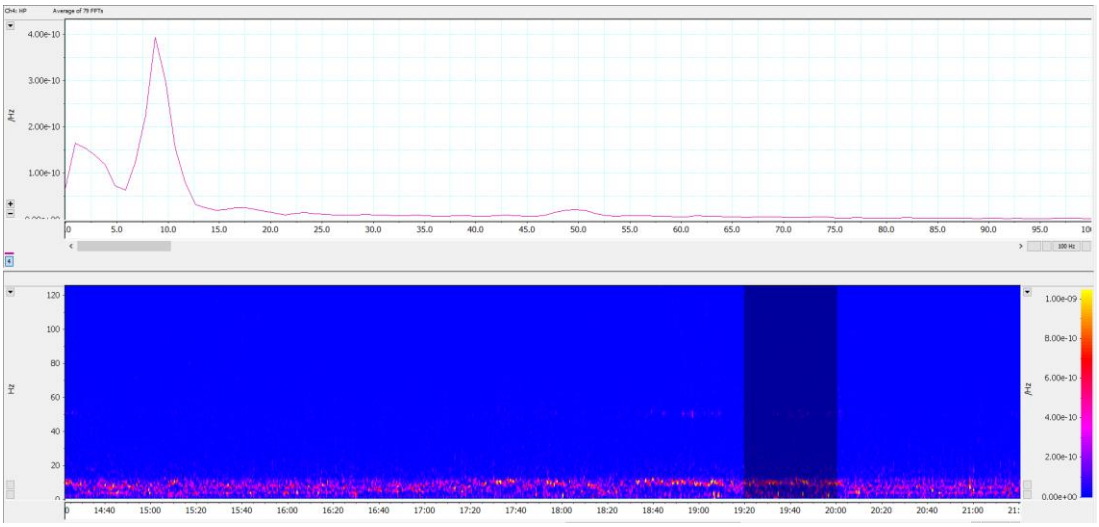

Figure 2.7 PSD and spectrogram pane in LabChart software

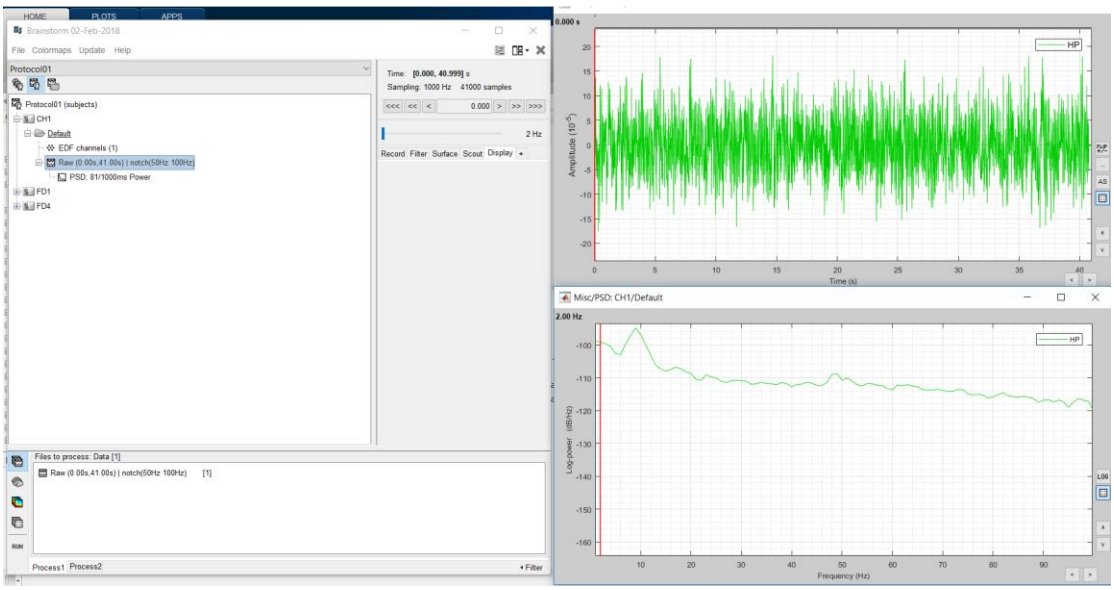

Figure 2.8 PSD calculation in Brainstorm toolbox

2.4.2 Spectral coherence analysis

Coherence indexes of electrode pairs were computed by Brain storm toolbox to localize the linked synchronous activity and long-range connectivity between two different regions during the task. Preprocessed signal data imported to the toolbox were analyzed by the selection of each electrode pairs (LHa-NAc, LHa-HP, LHa-OB, NAc-HP, NAc-OB and OB-HP) in the options of pipeline editor. Magnitude-squared coherence measure was performed for individual coherence outputs at time window for 100 Hz highest frequency of interest (Fig 2.9). The averages of value in each group of treatment were undertaken for frequencies 1-100 Hz, and at the particular ranges of seven frequency bands.

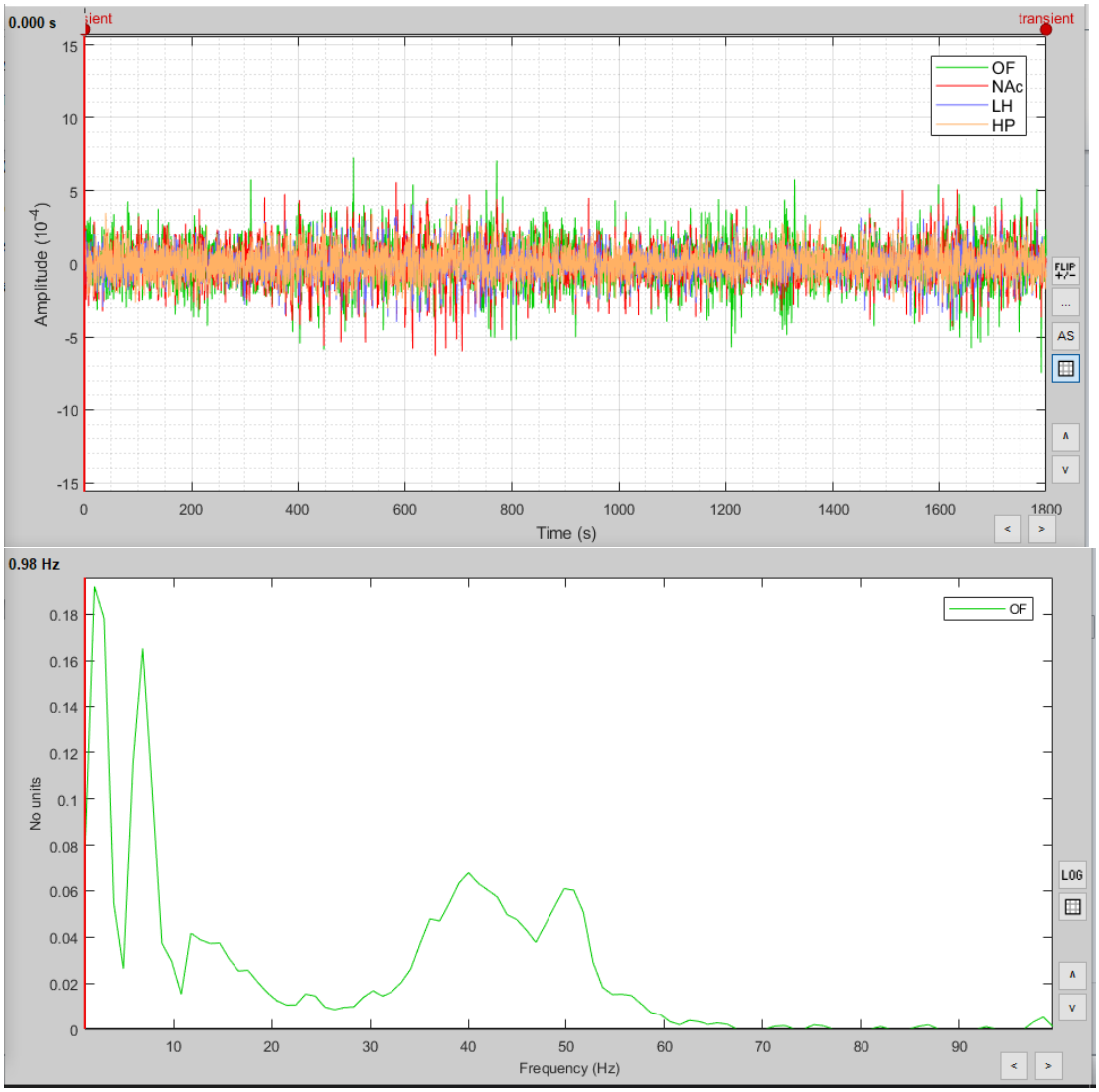

Figure 2.9 Example of coherence result analysis of signals from 4 regions by using Brainstorm toolbox

2.4.3 Phase-amplitude coupling (PAC) analysis

The preprocessed signal from a particular region was obtained for phase-amplitude coupling analysis by using Brainstorm. The generation of comodulogram and extraction of phase relation at a particular frequency coupled to higher frequency amplitude was analyzed. In this study, the nesting frequency band, in this case of interested low frequency, was screened from 1 to 12 Hz, and nested frequency band known to be the higher frequency was estimated from 30 to 100 Hz. The comodulogram contained many parameters of modulation index (MI) that are

maximal modulation index and frequency at the highest coupling. Color code can display the degree of coupling (Fig 2.10).

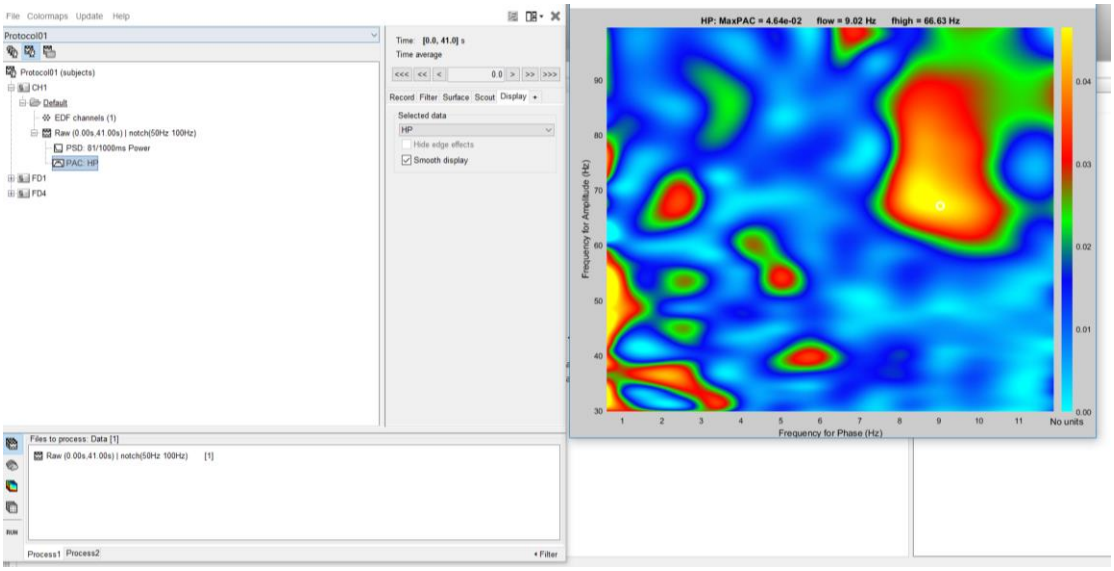

Figure 2.10 Phase-amplitude coupling analysis and comodulogram in Brainstorm toolbox

2.5 Behavioral Scores and Locomotor Activity

For general behavior observation, animals had their movement recorded by camera recorder on the top of experimental apparatus during the experiments. The useful custom-made automated tracking software was developed to quantify animal movement reliably with the qualities of high spatial and temporal resolution (Cheaha et al., 2015). The software makes more robust to tracking distance, speed and number of movement from video input that the digital video based-tracking system was written to follow up the white color animal body contrasted from the black color background of the apparatus. The animals' paths tracking allowed calculating distance traveled, continuous movement between 2 stops as a number of movement and distance traveled per unit time as traveled speed. The sensitivity of tracking from one location to another was set to 2-mm threshold.

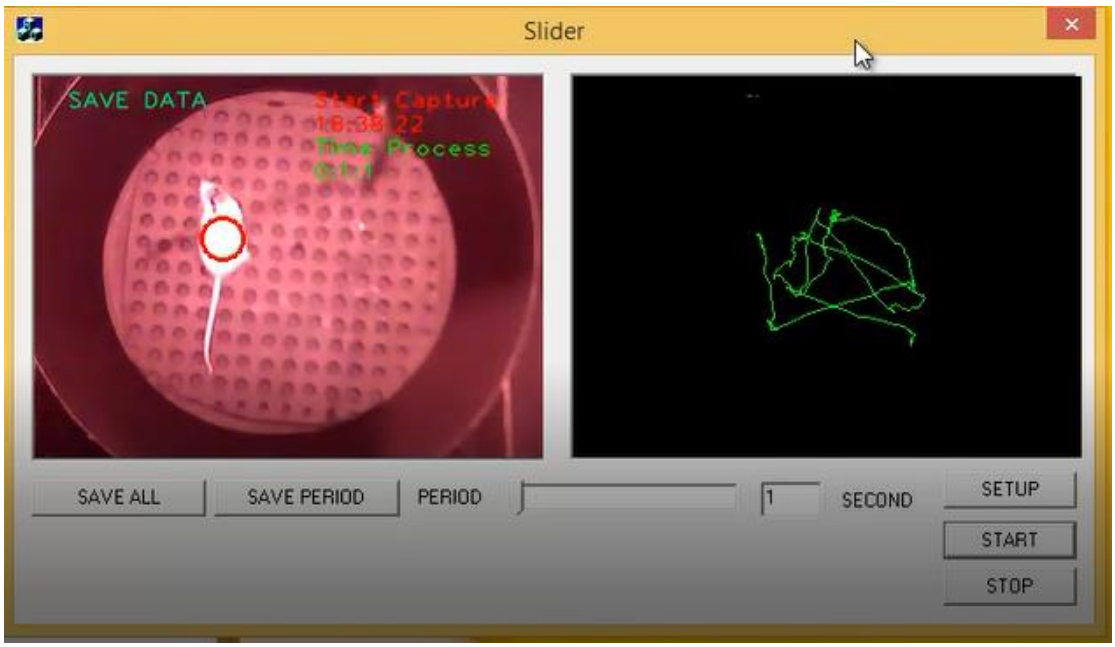

Figure 2.11 Custom-made automated locomotor tracking software. Red circle is set to track center of animal based on the contrast of white animal body to black background of apparatus. Green line represents animals' paths during the experiment allowed the calculation of a broad range of information about the locomotion of animal such as the frequency and time spent of movement.

2.6 Blood Glucose Measurement

In this study, increased or decreased energy level might influence to neural activity and behavioral changes. Then, plasma glucose from tail vein of eight animals at normal resting, fasting to induce hunger and following feeding in induce satiety were measured by using glucose meter (Accu-Check Acine, Roche) twice for before observation and 20 minutes after manipulation of oral gavage. The relative changes were determined in this study. The liquid diet in this study fed to induce satiety was prepared freshly by 2 g chow powder with 2 ml drinking water. The mixture was blended before feeding to animals.

2.7 Statistical Analysis

All analyzed results were expressed in mean values with standard error of mean (S.E.M.). A paired t-test was applied to determine the significant differences of the mean in two sets of data arrived from the same individual. Unpaired Student's t-test was used in some cases to determine statistical difference between two

independent data sets. To interpret the key results, one-way repeated measure ANOVA was used to report the significant difference of mean scores collected from different conditions in the same individual. The frequency bands and their analyses for the consideration of two influent factors were analyzed by using two-way and two-way repeated measures ANOVA. Statistical significant differences were considered to significance at p - value < 0.05.

CHAPTER 3
THE NEURAL NETWORK ACTIVITIES IN THE BRAIN REGIONS ASSOCIATED WITH HOMEOSTATIC HUNGER

Most of current research on eating has focused on identifying eating-related brain pathways that control feeding in a modern lifestyle. There are scientific reports suggesting many brain regions working in coordination as networks. However, not so many studies have investigated how the brain regions connect with each other to form the functional brain network architectures in response to hunger and fullness. In this study, the LFPs were altered in the hunger brain center (LHa), reward related brain (NAc), the brain area of learning and memory process (HP) and area of olfaction (OB). The interrelations among them were observed during a period of hunger and fullness assigned by overnight fasting and fluid food forced feeding, respectively, in mice. Therefore, associated neural signaling from the brain regions correlated with levels of energy balance in animals would complete a picture of common brain mechanism of emotional drive to eat. Notably, this neural network of homeostatic eating did not primarily respond to plasma glucose influence indicating the implement of mysterious CNS function in regulation of food intake.

3.1 Experimental Protocols

In the study of correlation between homeostatic hunger and brain signaling, fifteen animals were habituated in the black cylindrical apparatus half an hour per day for 3 consecutive days to acclimatize with the environment of recording. The experiments were performed in night time around 6 to10 p.m. because of the duration of aberrant foraging pattern. There were three conditions varying with respect to levels of energy balance in the body; negative, positive and normal energy status. Negative energy status was induced by 18-20 h food deprivation intended to produce hunger sensation. 0.1 ml fluid food feeding per 10g body weight was enforced to

animals to induce satiety. In control condition, animals were fed with the same volume of drinking water. Dummy, fluid food and, water feeding were carried out by gently inserting a ball-tipped stainless steel needles directly to stomach. The gavages were given to animals before obtaining in the experimental apparatus. Three different conditions of energy were randomly assigned to each animal within 3 consecutive days. Therefore, LFPs and locomotor activity were recorded for 30 minutes thereafter. LFP signals and behavior monitoring at 20-25 minutes of records were analyzed.

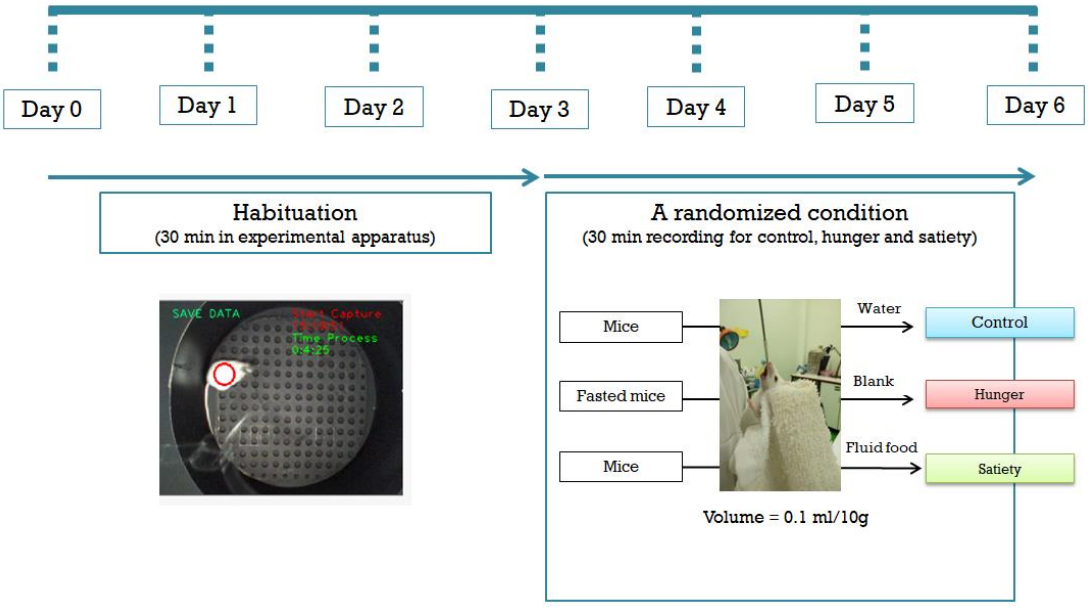

Figure 3.1 Laboratory experimental protocol for neural network activities in the brain regions associated with homeostatic hunger

3.2 Frequency Analysis in Brain Regions Associated with Homeostatic Hunger

The raw LFP traces (1-200 Hz) during control, hunger period and satiety period were presented in Fig 3.2. Different patterns of LFP signals were observed by visual inspection. In comparison to control, LFPs from the regions during hunger period showed higher frequency activity. Basically, properties of slow oscillation show the large amplitude waves in tracing. On the other hand, small amplitude oscillation can be described for the presence of high frequency.

Mean power spectrums of each frequency bands in particular region were statistically analyzed. The analysis showed some similarities of PSD change found among the regions of LHa, NAc and HP. No power density change was observed in

the OB (Fig 3.3 A). The increased power of synchronization in high beta ($F_{(2, 44)} = 4.179$, $p=0.026$) and high gamma ($F_{(2, 44)} = 5.546$, $p=0.009$) ranges were observed in Accumbens (Fig 3.3 B). Significant changes of LFPs in the LHa were reported in the frequency ranges of low beta ($F_{(2, 44)} = 5.506$, $p=0.010$), high beta ($F_{(2, 44)} = 5.061$, $p=0.013$) and high gamma ($F_{(2, 44)} = 5.918$, $p=0.007$) (Fig 3.3 C). Similar patterns of PSD in neural signaling of hunger seen in the HP with significant differences in high beta ($F_{(2, 44)} = 5.835$, $p=0.008$), low gamma ($F_{(2, 44)} = 4.423$, $p=0.021$) and high gamma ($F_{(2, 44)} = 7.432$, $p=0.003$) (Fig 3.3 D).

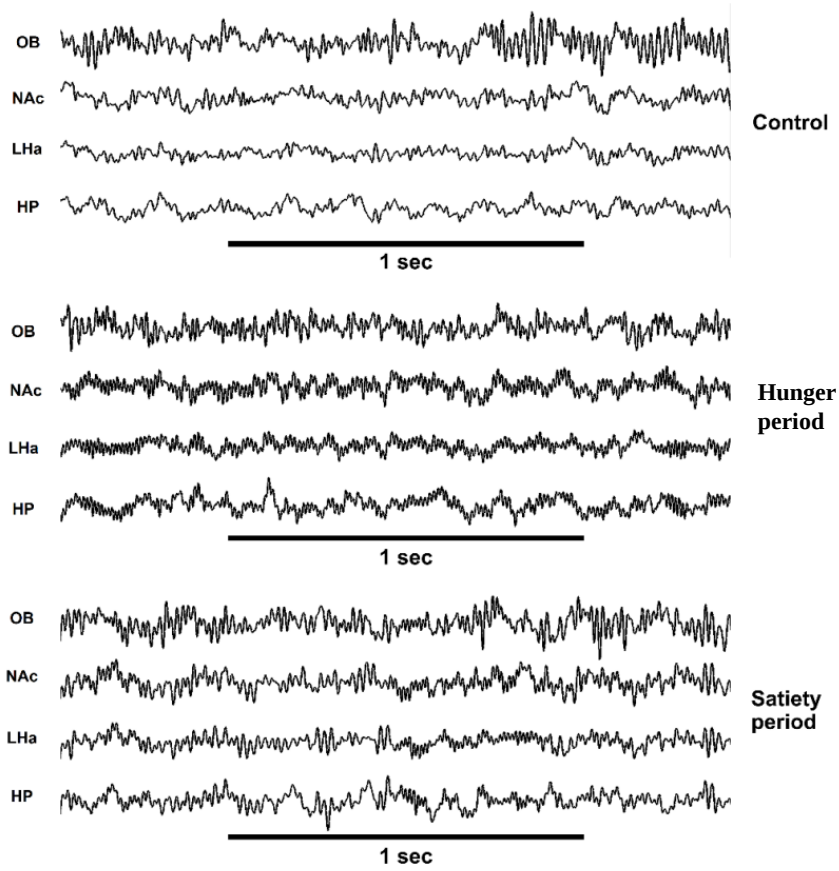

Figure 3.2 Raw LFPs collected from OB, NAc, LHa and HP on influencing of energy balance; resting control, hunger and satiety periods.

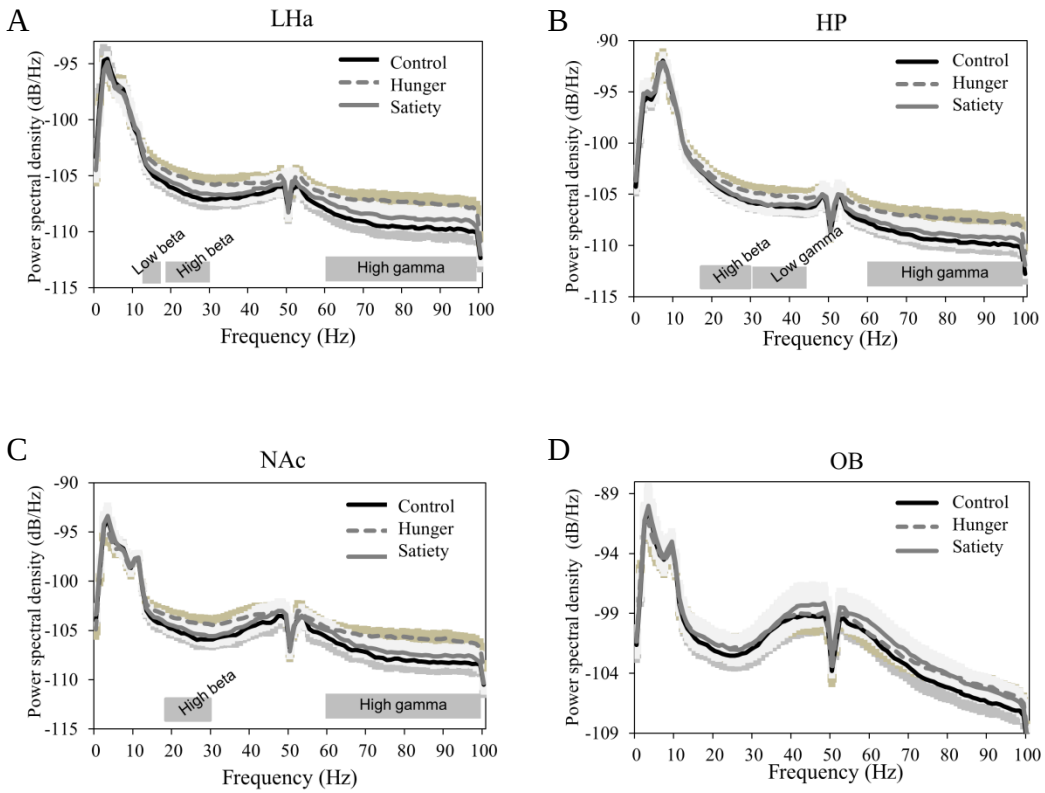

Figure 3.3 Power spectral density plot of control, hunger and satiety period overlaid according to the regions of lateral hypothalamus (A), dorsal hippocampus (B), nucleus accumbens (C) and olfactory bulb (D). Data were averaged and expressed as mean ± S.E.M. The influences of conditions were determined by using one-way repeated ANOVA followed by Tukey's *post hoc* test. Gray highlight indicates the frequency ranges significantly at $p<0.05$.

3.3 The Coherence Analysis for Hunger

Functional connectivities of phase synchrony between pairwise LFP signals were analyzed. Comparisons of coherence patterns of hunger and following feeding to satiety were relative to resting control (20-25 minutes after recording). Significant differences were observed in averaged coherent activity in the LHa- HP interrelation. One-way ANOVA repeated measure and multiple comparison revealed ranges of high beta ($F_{(2,\ 44)} = 9.8011$, $p<0.001$) and low gamma ($F_{(2,\ 44)} = 6.735$, $p=0.004$) were increased in hunger period (Fig 3.4A). No significant influence in LHa- HP coherent activity was produced by satiety. On the other side, the coherence synchronization in the LHa interplayed to the NAc had activity patterns in the decrease of low gamma (F

$_{(2,\ 44)} = 4.332$, p=0.023) observed in mean coherence value induced by hunger (Fig 3.4B). No significant observation in LHa-NAc coherent activity was produced by satiety. In addition, all frequency activity exhibited neither hunger nor satiety influences affected to the LHa-OB coherent activity (Fig 3.4C).

High impact of negative energy status were noticed in the increased NAc-OB coherence in frequency oscillations at low beta ($F_{(2,\ 44)} = 11.948$, p<0.001), high beta ($F_{(2,\ 44)} = 9.376$, p<0.001) and high gamma ($F_{(2,\ 44)} = 15.001$, p<0.001) ranges (Fig 3.5A). During satiety, the coherence increase in low beta band was also similar pattern which observed as in hunger condition (Fig 3.5A). The interrelative NAc-HP coherence that animals were food deprived was confirmed the decrease in a frequency range of low gamma ($F_{(2,\ 44)} = 4.680$, p=0.018) (Fig 3.5B). By satiety, the NAc-HP coherent activity was not influent (Fig 3.5B). No significant difference in OB-HP coherent activity was induced by either hunger or satiety conditions (Fig 3.5C).

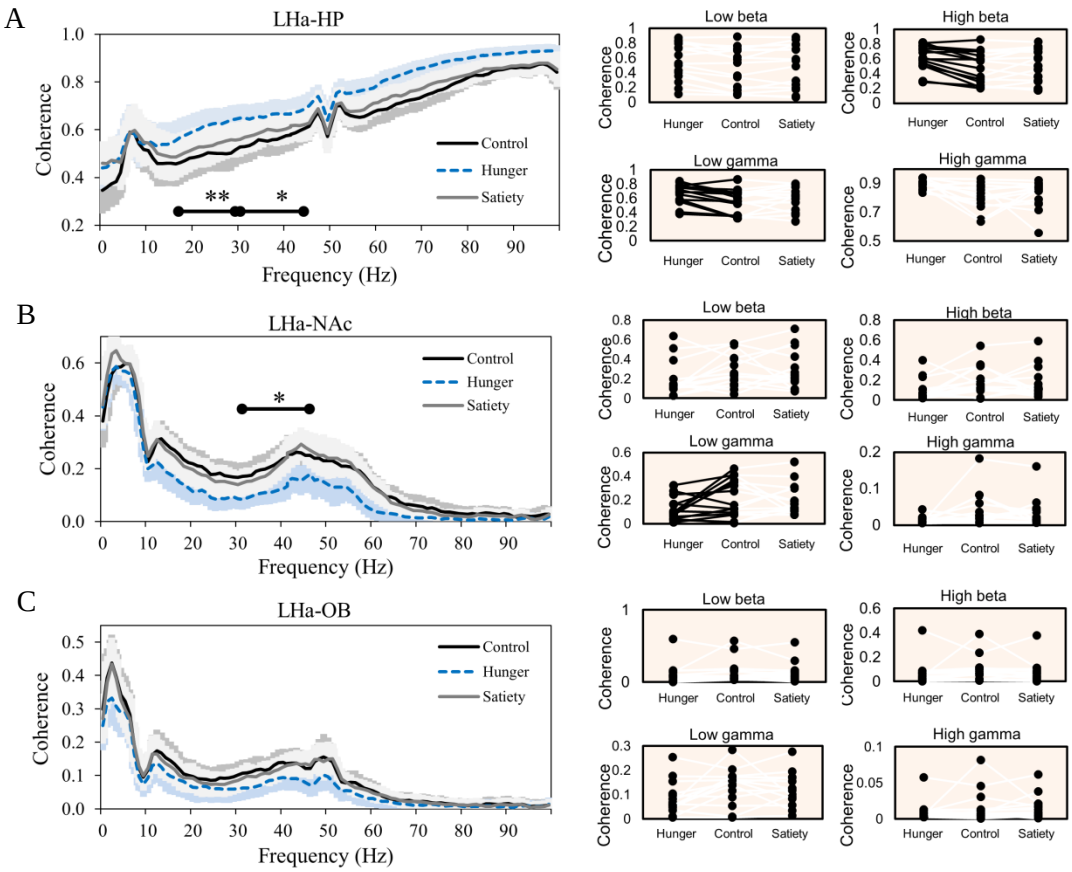

Figure 3.4 Mean coherence among LHa-HP (A), LHa-NAc (B) and LHa-OB (C) over 7 frequency bands during control, hunger for hunger and feeding to satiety. Data were averaged and expressed as mean ± S.E.M. The influences of conditions were determined by using one-way repeated ANOVA followed by Tukey's *post hoc* test. $\overset{*}{\leftrightarrow}$ indicates the frequency ranges significantly at $p<0.05$, and $\overset{**}{\leftrightarrow}$ indicates the frequency ranges significantly at $p<0.001$. Black dots represent individual coherence values in different conditions.

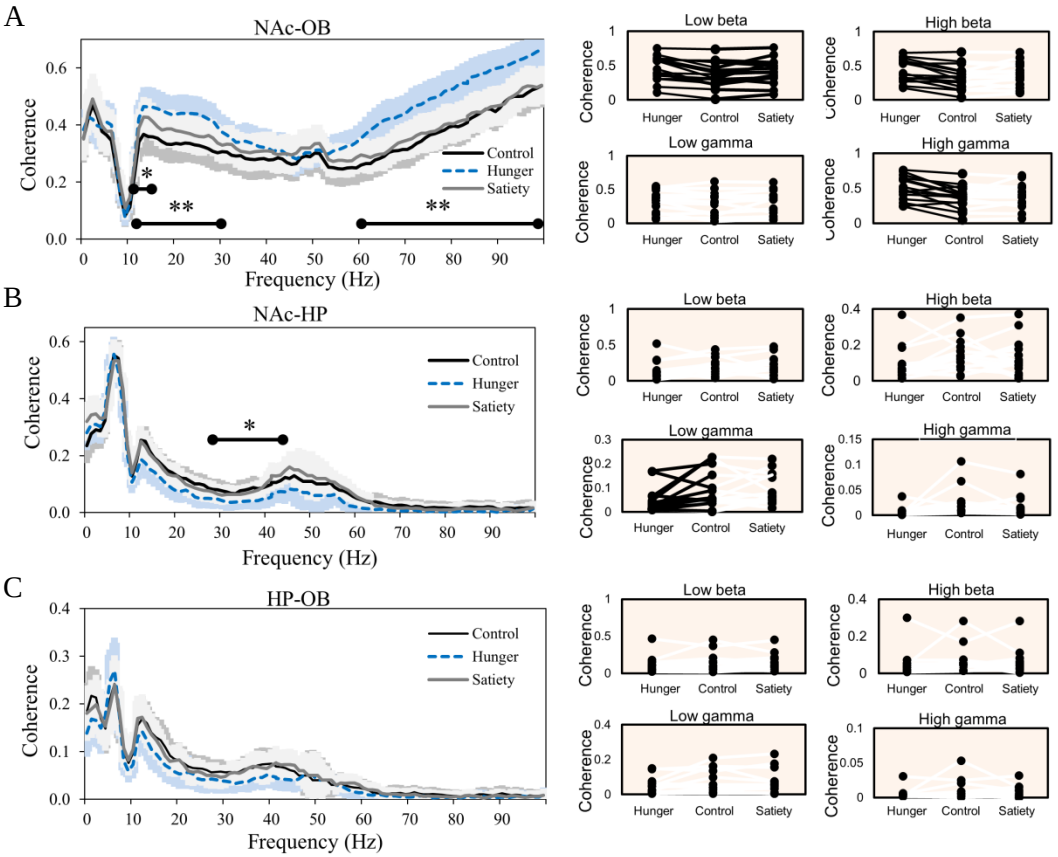

Figure 3.5 Mean coherences between the pairs of NAc-OB (A), NAc-HP (B) and HP-OB (C) over 7 frequency bands during control, fasting for hunger and feeding to satiety. Data were averaged and expressed as mean ± S.E.M. The influences of conditions were determined by using one-way repeated ANOVA followed by Tukey's *post hoc* test. $\overset{*}{\leftrightarrow}$ indicates the frequency ranges significantly at $p<0.05$, and $\overset{**}{\leftrightarrow}$ indicates the frequency ranges significantly at $p<0.001$. Black dots represent individual coherence values in different conditions.

3.4 Plasma Glucose Measurement and Locomotor Evaluation

To determine plasma glucose as a consequence of food deprivation and food satiation, blood glucose from tail vein of animals were measured before and 20 minutes following gavage feeding of fluid food for satiety, water feeding for control and empty gavage (without food/water) for hunger condition. One-way repeated measures ANOVA revealed significant difference in glucose levels among treatments ($F_{(2,\ 23)} = 7.263$, $p=0.007$). Multiple comparisons indicated a significant difference

between hunger and satiety conditions (Fig. 3.6A). However, no significant alteration was produced neither by hunger nor satiety when compared with glucose level of control group.

Locomotor activity was analyzed in terms of horizontal movement activity, distance travelled and time spent on moving. Each locomotor value was averaged and compared among the three conditions. One-way repeated measures ANOVA depicted no significant difference in these parameters during hunger or satiety periods (Fig 3.6B-D).

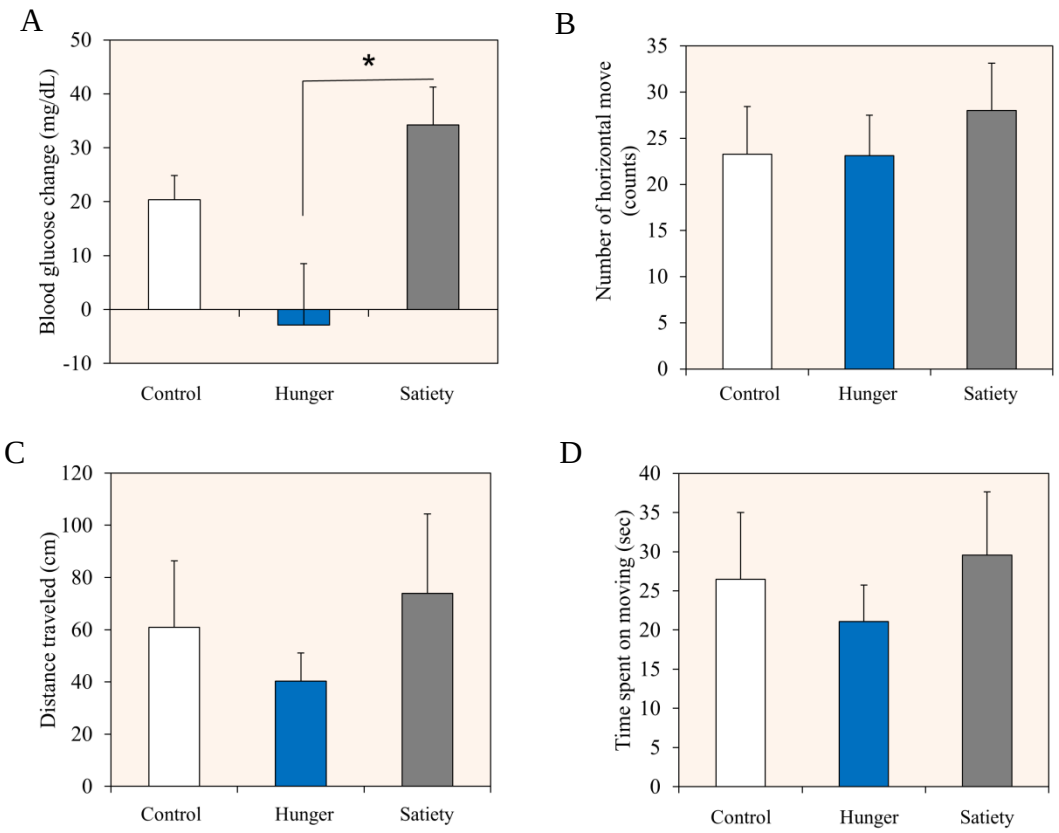

Figure 3.6 Plasma glucose levels change (n=8) and three parameters of locomotor activity (n=15) during control, hunger and satiety. Influences of conditions were determined by one-way repeated measure ANOVA, and * indicates the significant difference at $p<0.05$.

3.5 Discussion and Conclusion

One of important findings in this study is that LFPs typically contain broad spectrum of frequency oscillations found to be directly related to brain rhythms and sensation of hunger and satiety in animal model. The LFP oscillation patterns have gained the attention to understand neuronal circuits reflecting how the brains work to regulate feeding. Magnitude-squared coherences were estimated for the pairs of LHa-HP, LHa-NAc, LHa-OB, NAc-HP, NAc-OB and HP-OB. Fasting as overnight in animals generated the neural signaling patterns specifically to enhance LHa-HP and NAc-OB connectivity. Food deprivation intended to induce hunger also decreased the coherence activity among LHa-NAc and NAc-HP. These were developed at specific frequency of beta and gamma ranges.

Power reflects the activity of the rhythm of the signal whereas coherence reflects the interrelation between activities of two different signals at specific frequency band in neural network activity. Hence, the increase of beta coherence could be defined together with a significant increase or decrease of signal power (Hipp et al., 2011). Synchronization of beta band (~12-30 Hz) was demonstrated to bind multiple sensorimotor areas into a large-scale network during motor maintenance behavior (Brovelli et al., 2004). Additionally, quantitative coherent analysis showed that the EEG and EMG coherence were enhanced at beta frequency specifically in motor performance indicating the effective corticospinal interaction (Kristeva et al., 2007). On the one hand, reduced beta coherence indices were noted as aberrant interhemispheric synchrony/asymmetry and a profile of frontal activation in male depression (Knott et al., 2001). As well as, cortical connection impairment in Alzheimer's patients was reported with the beta coherence decrease across temporo-parito-occipital regions and interhemispheric (Locatelli et al., 1998). Corticospinal beta coherence was necessary for the control of steady muscle contractions (Baker, 2007). In addition, neural activities in beta range also contribute to the transmission of olfactory signals to the hippocampal formation during odor sampling (noxious olfactory stimuli) (Chapman et al., 1998). This may explain one of functional role of beta coherent activity between the HP-OB in learning and memory for odor stimuli exposure.

Coherence of gamma-band oscillations are as basic processing between activities of neurons. Gamma coherence was generated among anatomically distinct interneurons, proposed to entrain the information inputs into gamma-coherent assemblies (Tort et al., 2007). In addition, gamma synchronization (30-80 Hz) was found to bind partially selective multiregional cortical areas for visual discrimination task in primate (Bressler et al., 1993). Previously, local sensory integration has been observed to correlate with the dynamic of gamma (~20-70 Hz), and multisensory integration has been evolved with the dynamic of beta (~12-18 Hz) (Von Stein and Sarnthein, 2000). Recently, coherence activity between regions within higher frequency ranges (beta and gamma) was studied in schizophrenic patients and found a significant decrease in coherence of both beta and gamma across the central and frontal regions accounting the failure of synchronous activity for cognitive functions such as working memory and executive process (Yeragani et al., 2006). Thereafter, there was a study depicting the synchrony activity in beta (22-34 Hz) and gamma (35-55 Hz) linked to top-down and bottom-up attentional processing respectively (Buschman and Miller, 2007). For the study in audiovisual task, beta (15-23 Hz) and gamma (74-97 Hz) coherence synchronization were enhanced in large-scale cortico-cortical network during stimulation presentation (Hipp et al., 2011). However, in a simplified model, rhythms in the beta and gamma ranges were found to have different dynamical structures and network or synchronization properties (Kopell et al., 2000).

The schematic diagrams were drawn to sum up the major findings of the present study for neural oscillatory response of hunger (Fig 3.7A) and satiety (Fig 3.7B). Robust findings of the present experiments in coherence activity responsible for fasting to produce hunger could be highlighted to neural oscillatory in circuits of key brain regions which included the hunger center (LHa), reward regions (NAc), memory integrated sites (HP) and the area of olfactory sensation (OB). These brain regions are important in receiving and integrating internal information inputs of hunger such as autonomic and endocrine systems. In particular, the LHa is found to be a brain major hub of neural integrating of forebrain control in feeding behavior and emotional state via autonomic and endocrine function (Saper, 2000). The electrical stimulation to the LHa can produce both eating and reward aspects as part of critical function of the hypothalamus with homeostatic, motivated behaviors and reward

process required for survival and reproduction (Berthoud, 2002; Margules and Olds, 1962; Sternson, 2013).

The molecular mechanisms underlying neural circuits implemented sensory processing, reward, hormone and motor movement process of food intake and energy homeostasis can bridge the LFPs findings as biomarkers of homeostatic hunger which the LHa, NAc, HP and OB represented the correlates in eating regulation (Berthoud, 2002). Previously, the physiological link between the regions of LHa and NAc was studied. It was found that glutamatergic receptor inhibition in the NAc induced feeding response as GABA agonist infusion in the LHa indicating the structural and functional links between these two major regions in ingestive behavior (Maldonado-Irizarry et al., 1995). Thus, the NAc is considered to reinforce or drive food intake during coping with homeostatic hunger via the projection to the LHa. Significant features of hippocampal oscillation can interpret mnemonic function for episodic meal-related memories and conditional learned associations between food-related stimuli and post-ingestive consequences to control feeding (Henderson et al., 2013; Higgs, 2008; Kanoski and Davidson, 2011; Kanoski and Grill, 2017c; Parent et al., 2014; Rozin et al., 1998b). Furthermore, olfactory system is one of the components that stimulate food intake both in human and animals. Previously, infants who received the specific scent of food during pregnancy showed the specific preference on the diet thereafter (Schaal et al., 2000). In addition, food pellet could induce Fos expression in cell layer of the OB, but sniffing response could be found during hunger in particular (Prud'Homme et al., 2009). On the other hands, olfactory bulbectomized rats altered the feeding patterns, and interrupted to the pathway of olfacto-hypothalamic system (Larue and Le Magnen, 1972; Meguid et al., 1993).

The physiological sensation of hunger has been critical for glucose level determination in the body and locomotor activity. Previously, blood glucose regulation may be considered to be a potential mechanism in the body to control hunger. In fact, the level is actually undertaken by the regulation of homeostasis especially to prevent hypoglycemia (Mayer, 1955). As the case of negative energy status, the plasma glucose level of fasted rats did not significantly change (Miselis and Epstein, 1975). Apart from that, the level could be increased in case of refeeding (Brecchia et al., 2006). Locomotor movement in eating behavior of animal model is

believed to relate with foraging for foods. There are several movement patterns in response to starvation by increased activity reflecting the wakefulness for food seeking (Cornish and Mrosovsky, 1965; Davenport and Evans, 1984; Yamanaka et al., 2003), and decreased movement as adaptive behavioral response for metabolic cost (Fu et al., 2011; McIntyre and Wiens, 1999; Wang et al., 2006). In the present results, distance traveled, locomotor count and speed were not affected by fasting or feeding. It could be explained due to no explicit food-related cue. Also, it would be beneficial to maintain energy for upcoming situation to reach foods.

In conclusion, beta (~12.5-30 Hz) and gamma band (~30-100 Hz) synchronizations in this study refer to the basic rhythms of connections which are distinct to neurons and brain architectures respectively. Thus, the network consisted of increased pattern of beta and gamma coherency across multiple regions may modulate the efficiency of information transmission. On the other hand, neural network activities during the period of hunger might undergo to enhance behaviors.

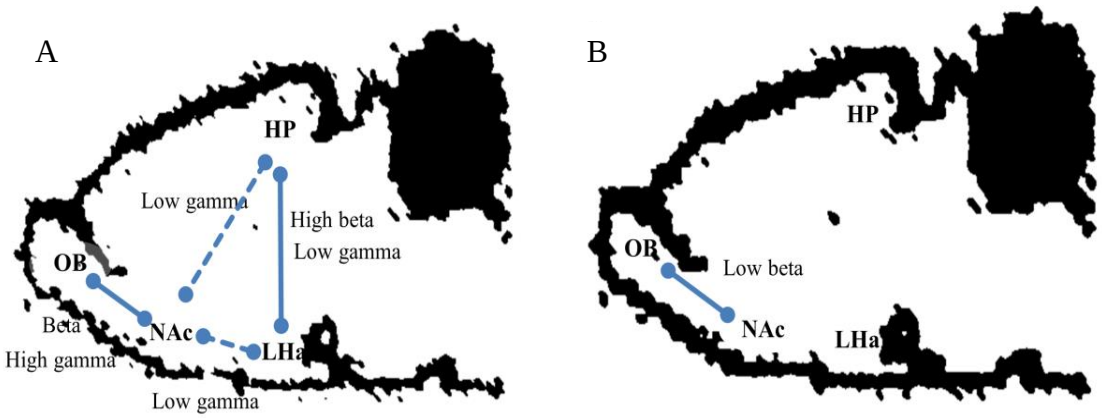

Figure 3.7 Brain schematic of neural network activities in association of levels of energy intake. Neural network activities during hunger (A) and satiety (B) were drawn. Solid lines represent increased coherence among regions and dot lines indicate decreased coherence among regions

CHAPTER 4
NEURAL SIGNALING FOR CHOCOLATE-LIKED BEHAVIOR

Eating motivation is induced not only by negative energy balance but also triggered by food cues. Moreover, numerous studies focus more on in the influence of food preference on eating habits independent of hunger. However, neural processing for acquisition of food preference remains to be established. This study was introduced to identify hippocampal neural signaling in satiated mice responding to olfactory cue (chocolate scent) after the completion of repetitive chocolate exposures. Hippocampal CA1 LFP signals and their relationship regarding to frequency changes across LHa-NAc-HP-OB circuit including exploratory behaviors of satiated animals were analyzed before and after a week of chocolate sessions. In laboratory study of behavior, chocolate session-treated group would be subjected to explore for chocolate during a period of fullness. This was to determine if they still search for chocolate compared to control group without chocolate session. Therefore, neural signaling in correlation with chocolate searching in satiated animals would be analyzed. This would allow to identify LFP oscillatory patterns of the brain circuit associated with emotional drive to eat palatable food. This would reflect brain mechanisms that underlie seeking behavior and overeating. Ultimately, the identification of emotional drive to eat might be important for early detection of eating disorders.

4.1 Experimental Protocol

Animals were assigned into two groups accordingly to the chocolate session treatment; chocolate-treated and control groups. Prior to the day of recording, a piece of 2 g chocolate once in animals' home cages is required to prevent any change that is a result of novel stimulus known as neophobia. At that time, animals were placed 30 minutes in the location of central zone of the place preference-liked apparatus daily

for 3 days. On the first testing, the walls blocked for 3 distinct zones were drawn up to release animals exploring the apparatus after animals were fed with 0.1 ml/kg body weight of fluid food implemented to satiety. After that, LFP and locomotor activity were recorded simultaneously for 30 min. Normal food chow and Hershey's creamy milk chocolate were covered; only scents of them could pass through the hole endowing animals incoming around the zone. The zones of food were random between 2 opposite sides of the chambers. To set up the chocolate session, a piece of 2 g chocolate was put in the home cages at day1, day3, day5, day7 after the first recording for chocolate-treated group. Control group did not receive chocolate in their home cages. On the second testing, LFP and exploratory behavior of satiated mice were measured again for the second time on day 8. Animals were fed food before recording. Data during a period of 20-30 min recording were analyzed. This protocol was to investigate changes in neural circuit activity induced by exposure to palatable food associated cue following repeated consumption.

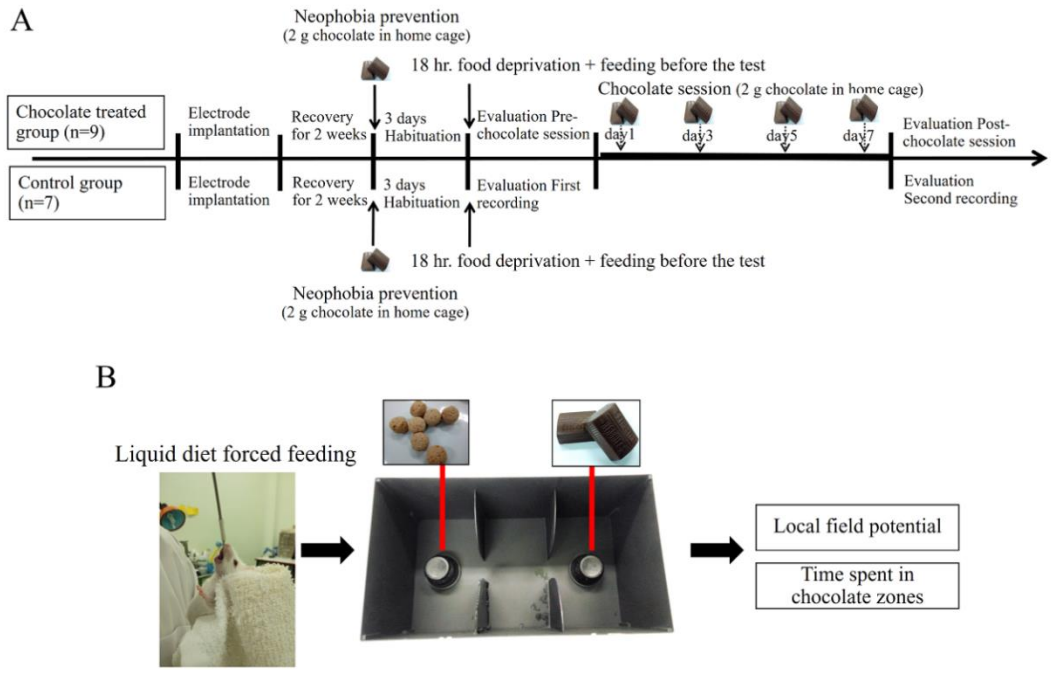

Figure 4.1 Experimental protocol (A) and feeding animals to satiety for chocolate preference observation (B)

4.2 Adaptive Configuration of Chocolate Preference and Hippocampal Function

4.2.1 Preference for chocolate and exploring behavior

Animals were forced fed with sufficient amount of fluid food to produce satiety before recording. Twenty minutes following forced feeding, blood glucose levels were found to significantly increase (p=0.029) (Fig 4.2B). Following chocolate sessions, chocolate treated-group exhibited preference for chocolate as seen from tracking system (Fig 4.2A). From locomotor tracking in second recording, animals entered and increased time spent in the chocolate zone [$F_{(1, 31)} = 5.578$, $p = 0.025$], but the speed of movement was not changed (Fig 4.2A, C, D). Time spent and numbers of travel in chocolate zone were significantly increased in chocolate session treated mice (Fig 4.3A, B). No behavioral change was observed in control animals.

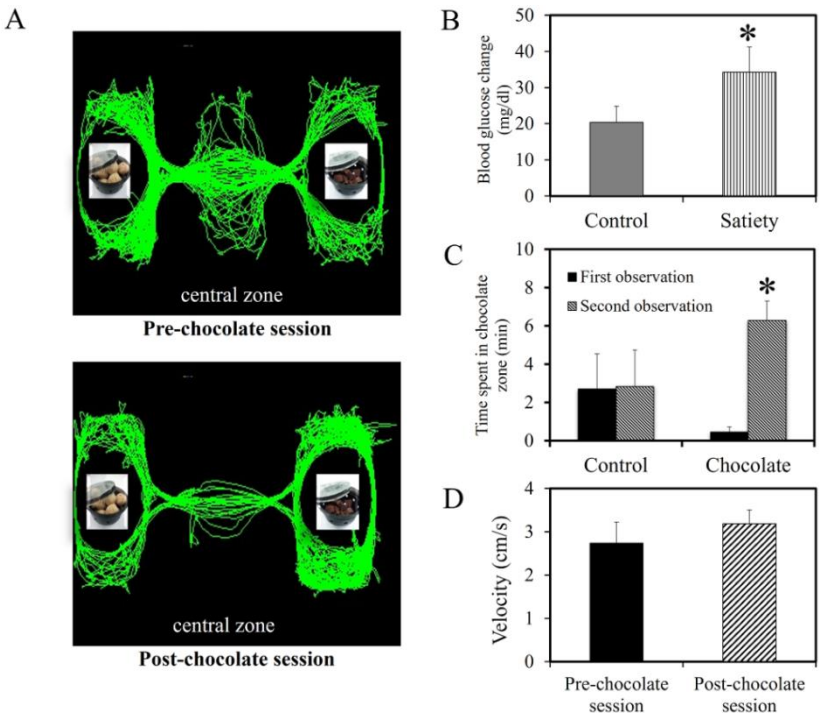

Figure 4.2 Blood glucose changes in mice (n=8) arranged of resting and feeding period, and exploring observation in food zones of control mice (n=7) and chocolate treated mice (n=9) and their locomotor indexes. The values were averaged and expressed as mean ± S.E.M. The influences of blood glucose changes between states and velocity between pre- and post-chocolate sessions were determined by using paired sample t-test, and two-way ANOVA followed by Tukey's *post hoc* test for factors of groups and treatments. * indicates the significant difference at $p<0.05$.

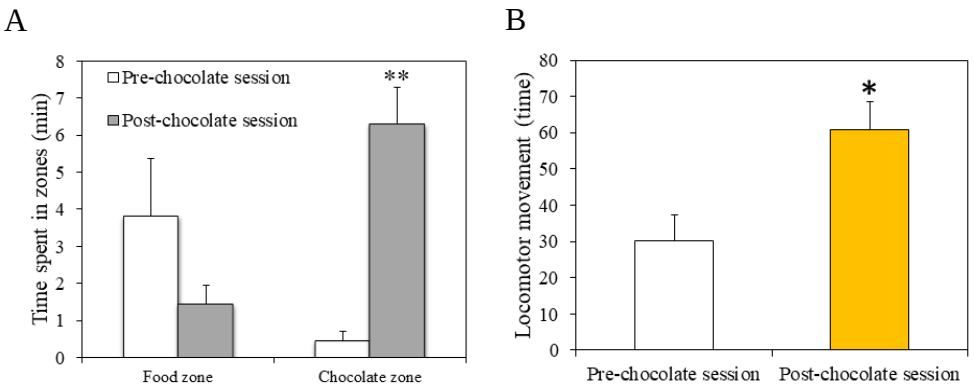

Figure 4.3 Locomotor evaluations of chocolate treated mice before and after chocolate sessions. The values were averaged and expressed as mean ± S.E.M. The influences of chocolate sessions were determined by using paired t-test, and two-way repeated ANOVA followed by Tukey's *post hoc* test were determined time spent in zones of chocolate-treated mice. * indicates the significant difference at $p<0.05$, and ** indicates the significant difference at $p<0.001$

4.2.2 Frequency analysis of LFPs following repeated chocolate intakes

Spontaneous LFP patterns in mouse hippocampus before and after chocolate sessions were reviewed by visual inspection for features of LFP tracings and spectrograms (Fig 4.4). At the second observation, the spectrograms of chocolate treated group were found to have intense theta activity and the LFP analysis in frequency domain displayed changes of value in percent total power. Fourier analysis showed the significant increase in power spectra of chocolate treated group at particular frequency ranges of delta and theta [$F_{(3, 71)} = 11.364$, $p<0.001$]. Significant changes in mean frequency power are indicated with bar graphs (Fig 4.5A, C). The power at corresponding peak frequency was increased for high theta frequency in comparison to that of pre-chocolate session (from 7 Hz to 9 Hz) (inset in Fig 4.5A). No significant of difference between first and second observations of control group was seen (Fig. 4.5B, D).

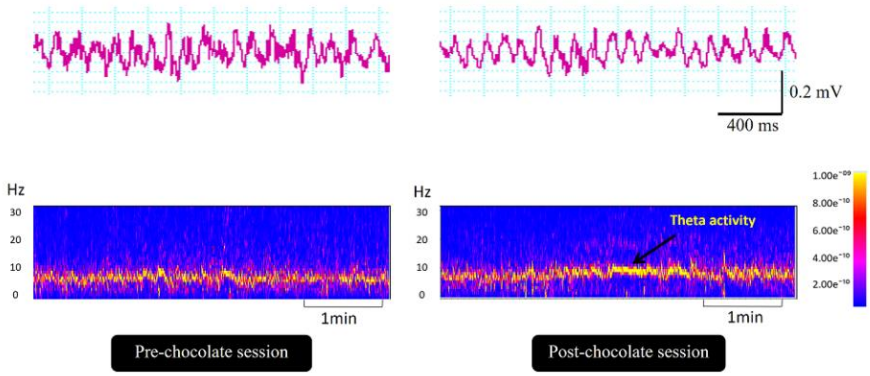

Figure 4.4 Spectral power before and after chocolate repeated consumption

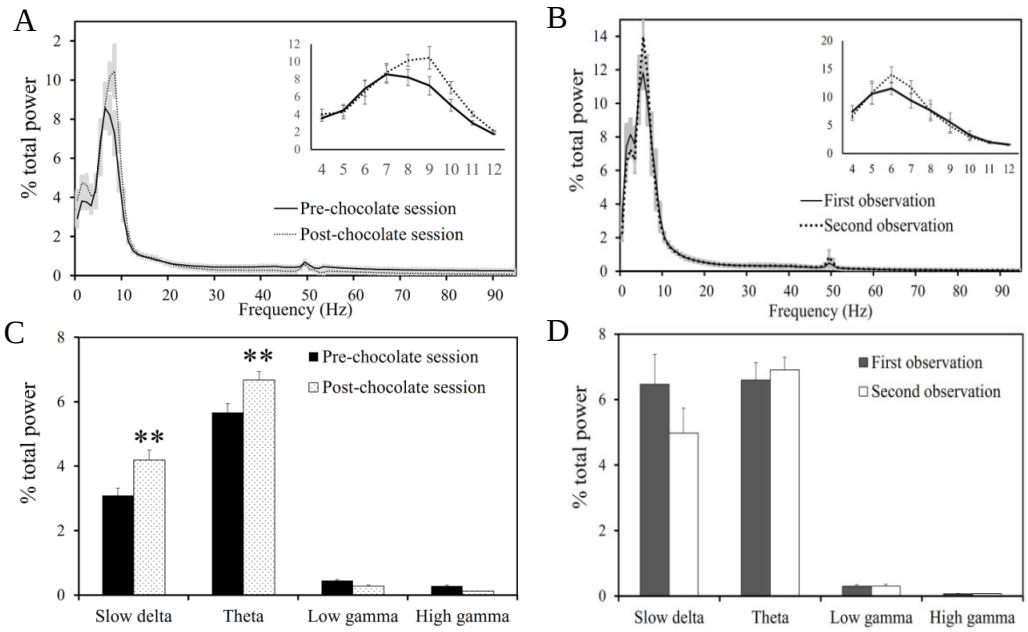

Figure 4.5 Percentage of total power in chocolate treated (A, C) and control (B, D) mice. Percent total powers were expressed as mean ± S.E.M. The influences of chocolate sessions were determined by using two-way repeated ANOVA followed by Tukey's *post hoc* test. ** indicates the significant difference at $p<0.001$.

Linear regression analyses were performed to determine the relationships between these oscillations and locomotor activity in term of maximal velocity during exploration in the recording chamber. The regression results revealed positive correlation between maximal power of low theta frequency (4-8 Hz) and maximal locomotor speed activity in post-chocolate session (R^2=0.24, P=0.04) (Fig 4.6C). On the other hand, maximal locomotor speed was not significantly correlated with total

power and maximal power of low theta frequency (Fig 4.6A-B). In fact, no significant change in mean of maximal power frequency in post-chocolate session compared to that of pre-chocolate session (Fig 4.6D).

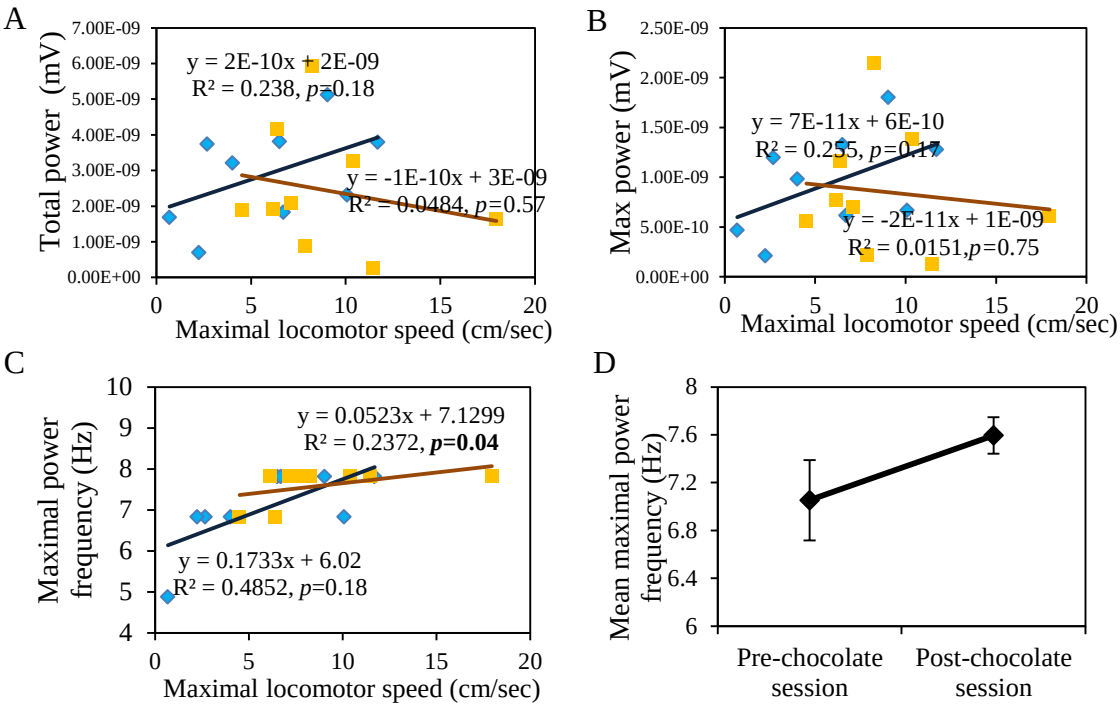

Figure 4.6 The regression analyses between hippocampal LFP low-theta oscillation and locomotor velocity at 20-30 min recording for exploration in place preference liked apparatus. Black lines indicate regression in pre-chocolate session and brown line indicate regression in post-chocolate session

Moreover, maximal locomotor speed was significantly correlated with total power of high theta frequency (8-12 Hz) during pre-chocolate session (R^2=0.48, P=0.04) (Fig 4.7A). There was no significant relationship between maximal locomotor speed and total power, maximal power and averaged maximal power frequency of high theta oscillation (Fig 4.7B-D).

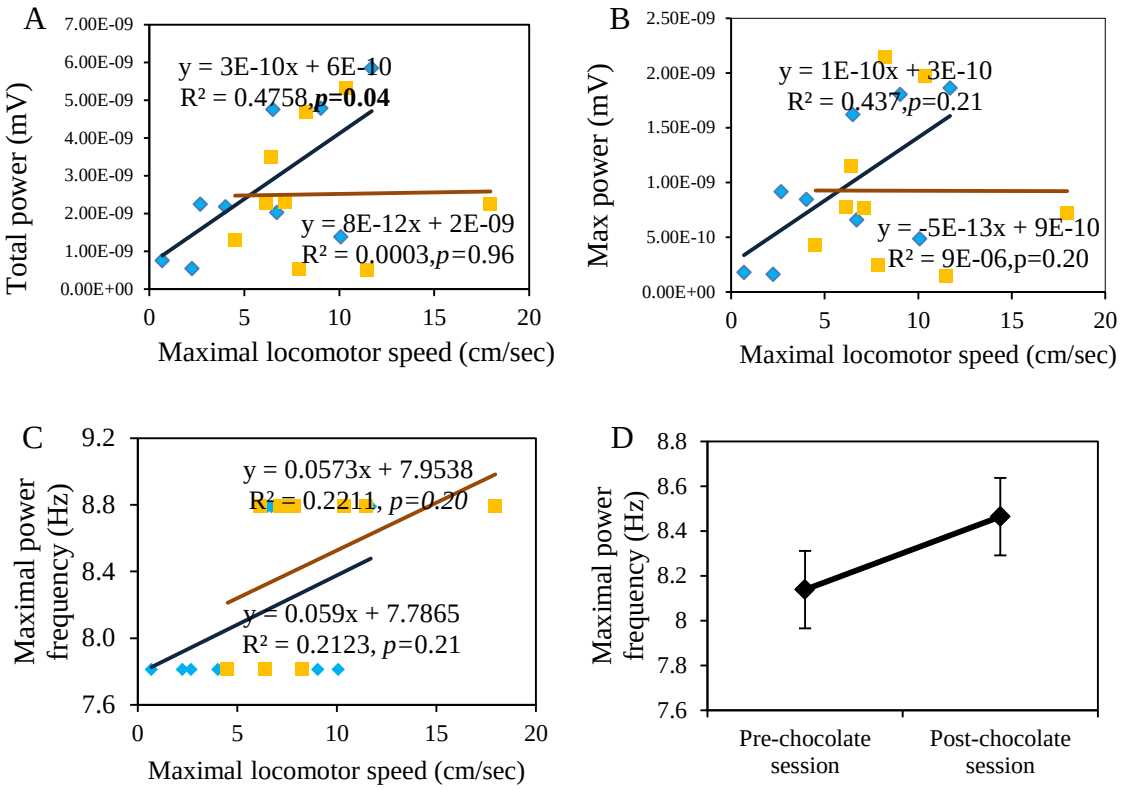

Figure 4.7 The regression analyses between hippocampal LFP high-theta oscillation and locomotor velocity at 20-30 min recording of exploration in place preference liked apparatus. Black lines indicate regression in pre-chocolate session and brown line indicate regression in post-chocolate session

Gamma frequency oscillation was investigated in correlation with speed of locomotor activity. Regression analyses revealed significant correlation between maximal locomotor speed and maximal frequency of low gamma wave (30-45 Hz) in post-chocolate session (R^2=0.47, P=0.04) (Fig 4.8C). No significant correlation was found between total power, maximal power and mean maximal power frequency (Fig 4.8A, B, D).

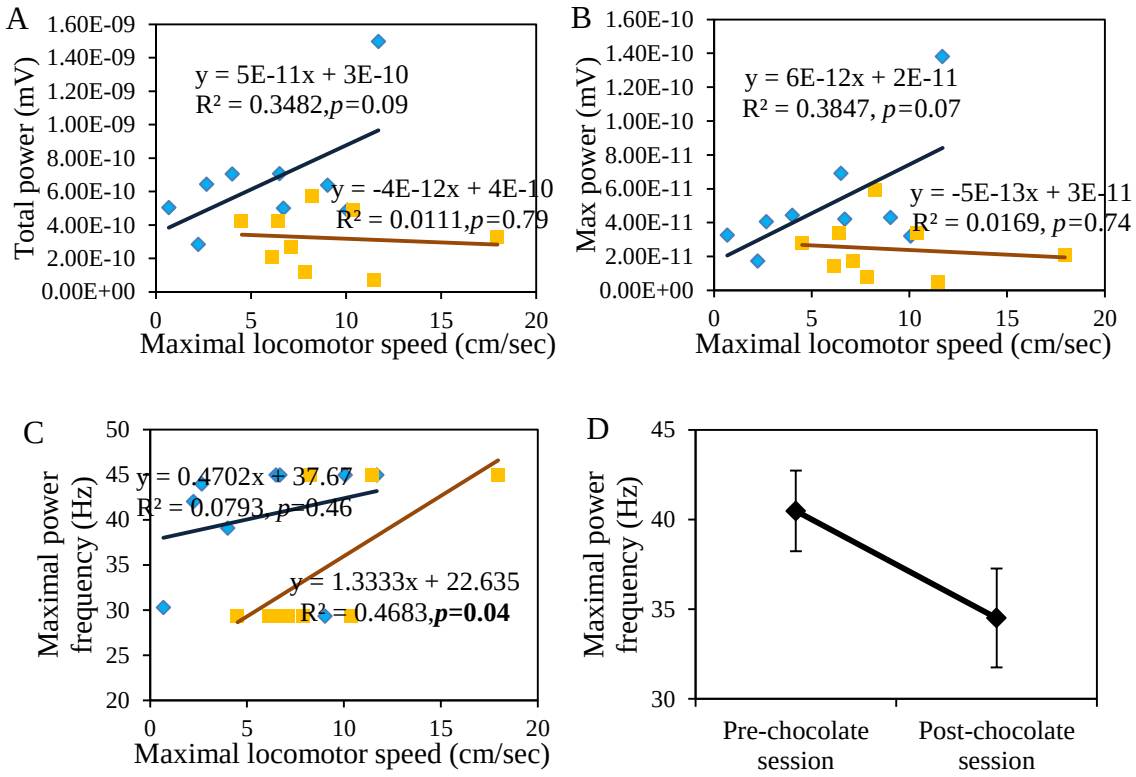

Figure 4.8 The regression analyses between hippocampal LFP low-gamma oscillation and locomotor velocity at 20-30 min recording for exploration in place preference liked apparatus. Black lines indicate regression in pre-chocolate session and brown line indicate regression in post-chocolate session

Moreover, high gamma oscillation (60-100 Hz) parameters were also tested with regression method in correlation with locomotor speed. The results showed that maximal power frequency in post-chocolate session was fixed at 64.5 Hz though maximal locomotor speed varied (R^2=1, P=N/A) (Fig 4.9A). Changes in maximal power frequency of high gamma range between pre- and post-chocolate sessions were tested by using paired t-test. The results showed that maximal power frequency was significantly decreased in post-chocolate session (p=0.028) (Fig 4.9D).

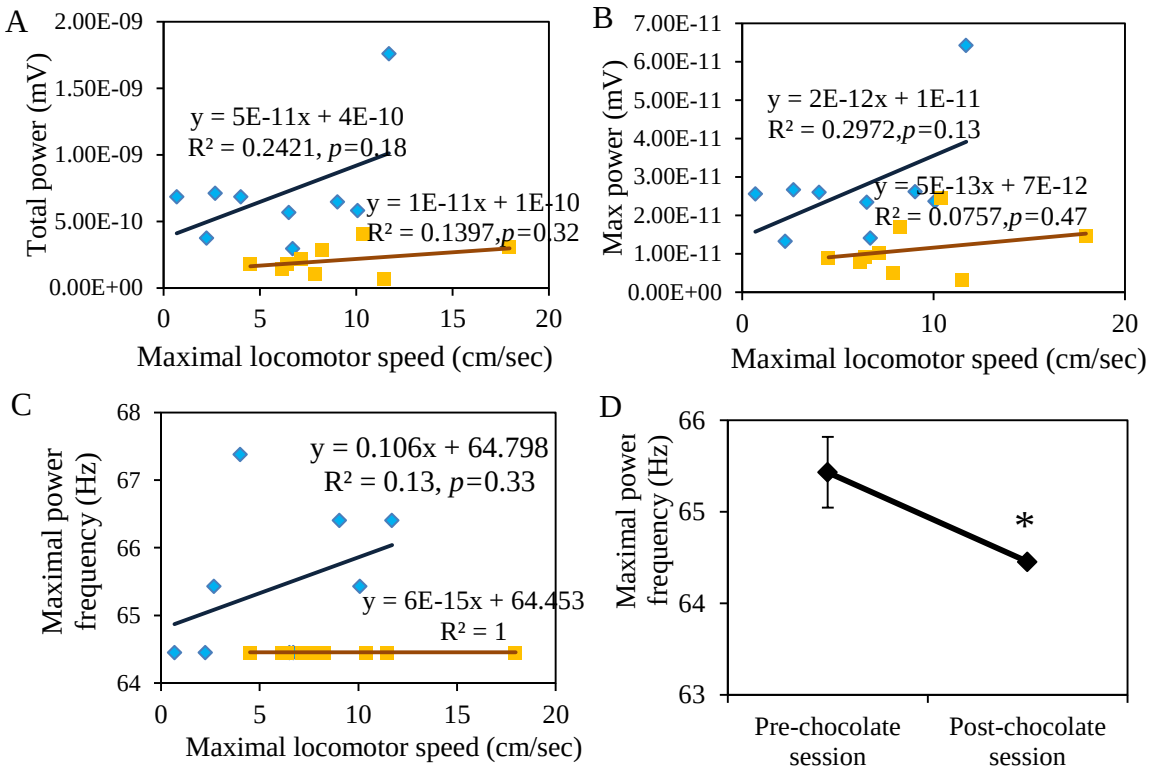

Figure 4.9 The regression analyses between hippocampal LFP high-gamma oscillation and locomotor velocity at 20-30 min recording for exploration in place preference liked apparatus. Black lines indicate regression in pre-chocolate session and brown lines indicate regression in post-chocolate session. * indicates the significant difference at $p<0.05$

4.2.3 Phase-amplitude coupling induced by chocolate sessions

In line with findings in behaviors, phase-amplitude coupling (PAC) analysis revealed the remarked strengthen of theta-gamma couplings of LFP signals recorded from CA1 region of the hippocampus during the second observation in chocolate session treated mice. Changes in the coupling of second observation were visualized in comodulograms (Fig 4.10A). The couplings were illustrated approximately at 7-9 Hz for phase and 60-80 Hz for amplitude. Maximal modulation index (MI) during the second observation of chocolate session treated mice was significantly increased compared to that of the first recording (p=0.021) (Fig 4.10B). Mean frequency for phase of maximal MI to modulate gamma oscillation was shifted to about 8 Hz

(p=0.003) in post-chocolate session (Fig 4.10C). However, the average of high frequency for amplitude did not change (Fig. 4.10D).

Delta and theta frequency bands were identified as nested oscillations of gamma sub bands amplitude. The index of theta rhythm modulated power of high gamma was significant elevated following post-chocolate session [$F_{(3,\ 71)} = 7.950$, p<0.001] (Fig. 4.11D-E). However, no significant difference was seen in theta-low gamma (Fig. 4.11C) and delta-gamma indexes (Fig. 4.11A-B). Between first and second observations, averaged PAC of control animals remain unaltered (Fig. 4.12A-E).

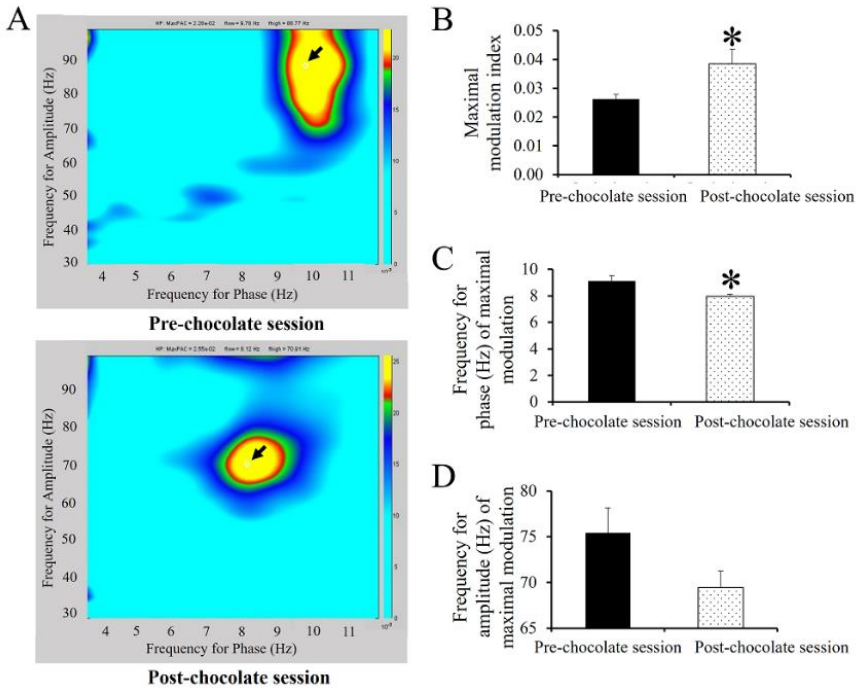

Figure 4.10 Nested theta and gamma oscillation coupling and its parameters measured in chocolate session treated mice. All data were averaged and express as mean ± S.E.M. The influences of chocolate sessions in chocolate-treated mice were determined by using paired sample t-test. * indicates the significant difference at p<0.05

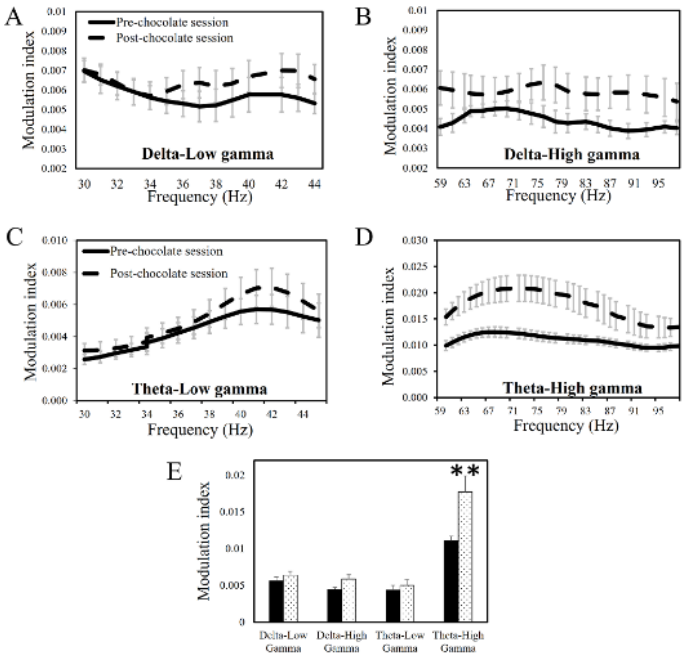

Figure 4.11 Averaged PAC in hippocampal region of chocolate-treated mice. Modulation index were express as mean ± S.E.M. The influences of chocolate sessions in chocolate-treated mice were determined by using two-way repeated ANOVA followed by Tukey's *post hoc* test. ** indicates the significant difference at $p<0.001$.

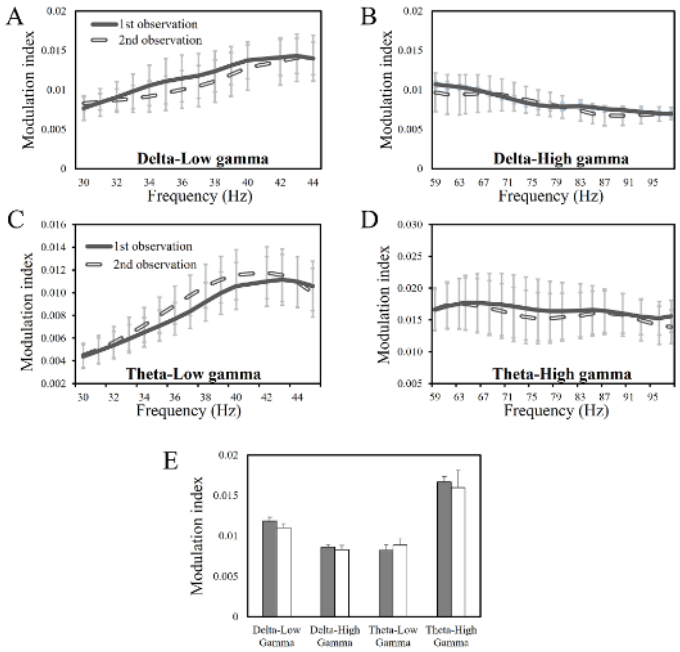

Figure 4.12 Averaged PAC in hippocampal region of control mice

4.3 Coherence and Network Oscillation Primes Food Approach Behavior

4.3.1 Adaptive estimation of power system of frequency

Raw LFPs in control and chocolate treated mice were taken 10 min time epoch of 20-30 min following the start of recording for the analysis (Fig 4.13). Changes in LFP oscillatory patterns following chocolate sessions were determined by using two-way ANOVA comparing between experimental chocolate treated and control groups and testing between values during the first and second observation. Spectral powers of each frequency wave statistically analyzed region-by-region. In the LHa, significant changes were induced following chocolate sessions [$F_{(1, 111)} = 21.443$, $p < 0.001$] (Fig 4.14A). Multiple comparisons also indicated significant increase in delta and decreases in high beta, low gamma and high gamma powers. In the NAc, significant changes were induced by chocolate sessions [$F_{(1, 111)} = 16.444$, $p < 0.001$] (Fig 4.14B). Multiple comparisons confirmed significant increases in delta and alpha, and decreases in high beta, low gamma and high gamma powers. Significant influence of chocolate sessions was also seen in the HP [$F_{(1, 111)} = 0.622$, $p = 0.432$] where multiple comparisons indicated significant increases in delta and high theta and decreases in low gamma and high gamma powers (Fig 4.14C). There was no significant change in the power analysis over frequency bands in the OB (Fig 4.14D).

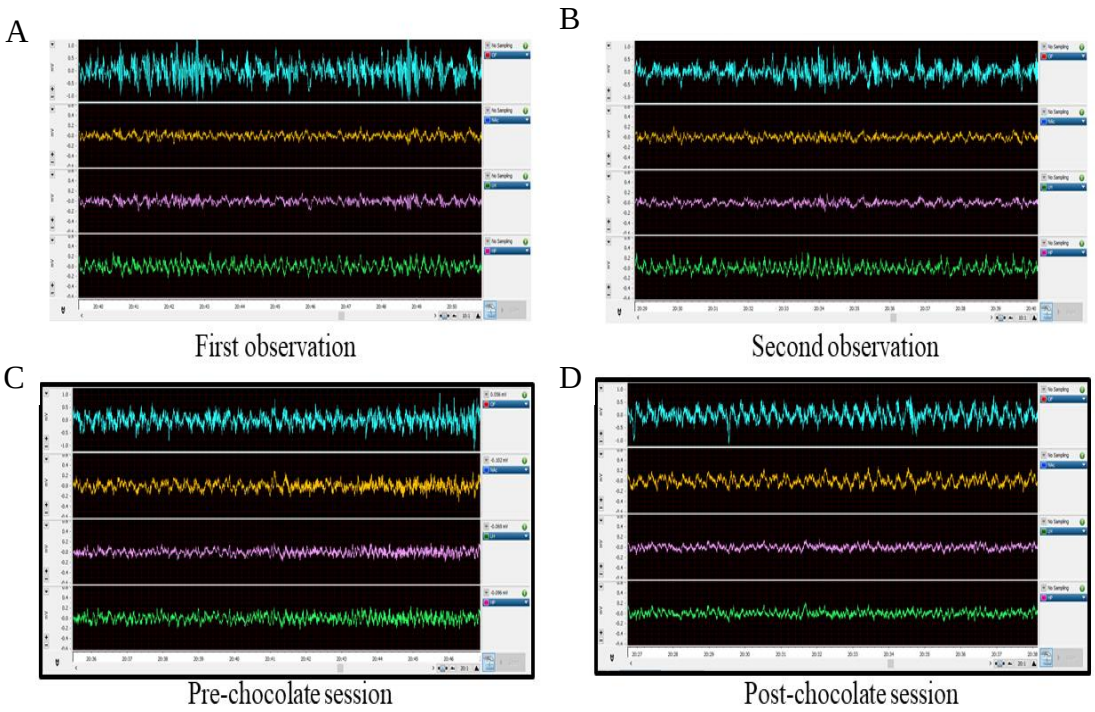

Figure 4.13 Raw LFPs recorded from 4 interested regions during exploration to food zones at 20-30 min. First and second observations were collected from control mice (A, B) and pre- and post-chocolate sessions were collected in chocolate-treated mice (C, D).

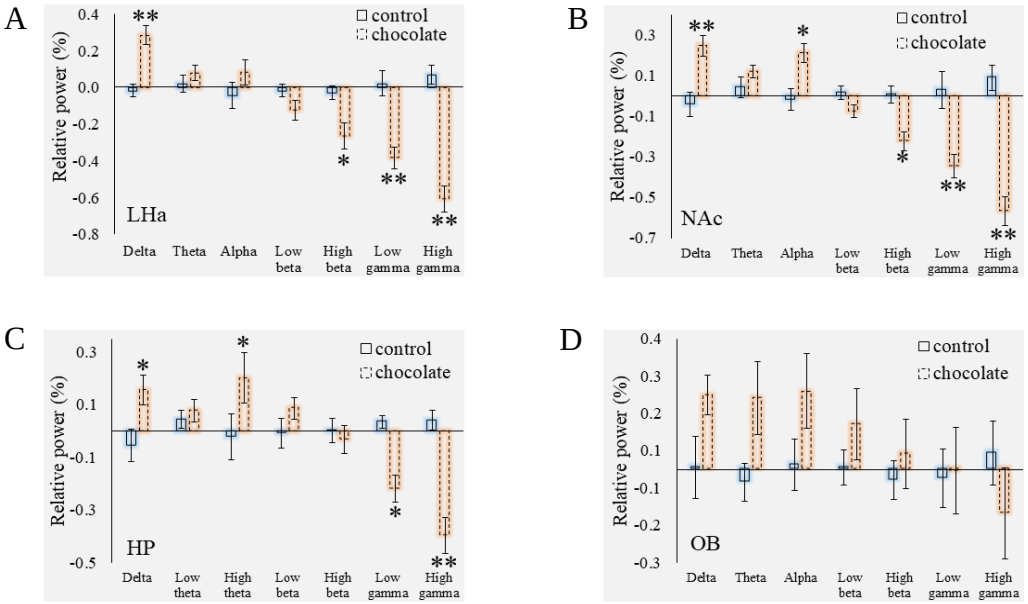

Figure 4.14 Percentage of relative power change in four brain regions; lateral hypothalamus (A), nucleus accumbens (B), hippocampus (C) and olfactory bulb (D). Data were averaged and expressed as mean ± S.E.M. The influences of group treatment were determined by using two-way ANOVA followed by Tukey's *post hoc* test. * indicates the significant difference at $p<0.05$, and ** indicates the significant difference at $p<0.001$

4.3.2 Coherence network

In order to evaluate whether how repeated chocolate consumptions affect the brain mechanism that underlies liking and wanting of forthcoming intake, comparisons of frequency coherence between pairs of brain regions during pre-chocolate session and post-chocolate session were determined. The percent of changes in coherent values were compared between the first recording and second recording in control mice.

Two-way ANOVA was used to determine significant influence of factors on coherent values in each frequency band. The results confirmed that chocolate sessions had significant effect on LHa-HP coherences [$F_{(1,\ 111)} = 17.419$, $p < 0.001$] (Fig 4.15A). Multiple comparisons (Tukey's post hoc test) indicated significant decreases in high beta ($p=0.04$), low gamma ($p=0.002$) and high gamma ($p<0.001$) (Fig 4.15D). Significant effects of chocolate sessions were found on spectral coherence between the LHa-NAc [$F_{(1,\ 111)} = 130.435$, $p < 0.001$] (Fig 4.15B). The post hoc test confirmed

significant increases in frequency ranges of alpha (p=0.009), low beta (p<0.001), high beta (p<0.001), low gamma (p<0.001) and high gamma (p<0.001) (Fig 4.15E). Interregional coherence increases by chocolate sessions were also found between the regions of LHa-OB [$F_{(1, 111)} = 77.486$, $p < 0.001$] (Fig 4.15C). Multiple comparison displayed the increases in alpha (0.038), low beta (p<0.001), high beta (p<0.001), low gamma (p<0.001) and high gamma (p<0.001) (Fig 4.15F).

Moreover, the patterns of NAc-HP coherence were significantly changed by chocolate sessions [$F_{(1, 111)} = 65.998$, $p < 0.001$] (Fig 4.16A). Significant changes between pre- and post- chocolate sessions were found in low beta (p=0.007), high beta (p<0.001), low gamma (p<0.001) and high gamma activities (p<0.001) (Fig 4.16D). Next, coherence activities between the pair NAc-OB were significantly changed by chocolate sessions [$F_{(1, 111)} = 6.066$, p=0.016] (Fig 4.16B). Significant changes between pre- and post- chocolate session were seen in low beta (p=0.039), low gamma (p=0.026) and high gamma activities (p<0.001) (Fig 4.16E). Finally, coherent activities between the pairs of HP-OB were also altered by chocolate sessions [$F_{(1, 111)} = 29.187$, $p < 0.001$] (Fig 4.16C). Significant changes in coherence of HP-OB interrelation were seen in high beta (p=0.025), low gamma (p=0.003) and high gamma activities (p<0.001) (Fig 4.16F).

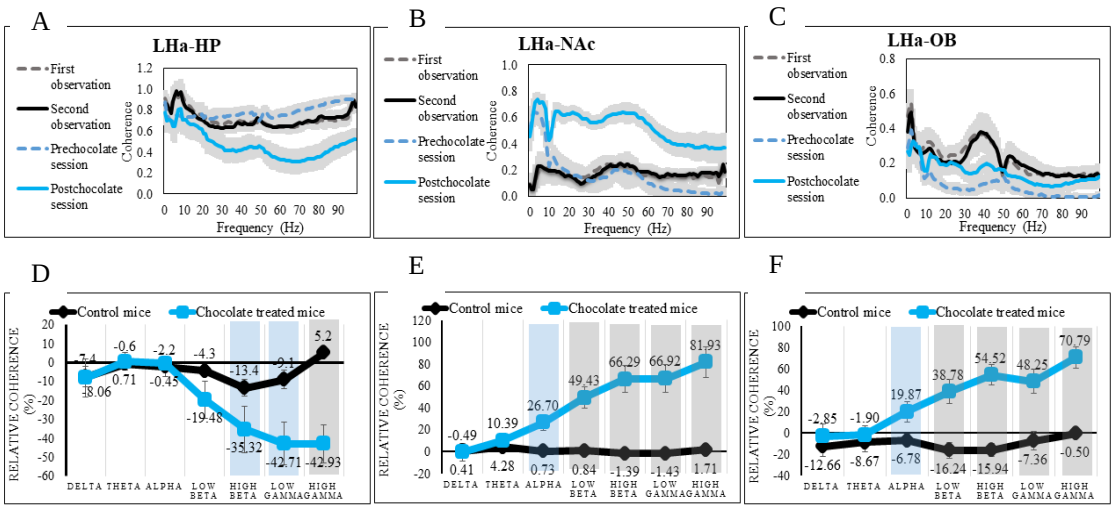

Figure 4.15 Interarial coherence differences in LHa-HP, LHa-NAc and LHa-OB. Data were averaged and expressed as mean ± S.E.M. The influences of group treatment were determined by using two-way ANOVA followed by Tukey's *post hoc* test. Blue marks indicates the significant difference at $p<0.05$, and gray marks indicates the significant difference at $p<0.001$

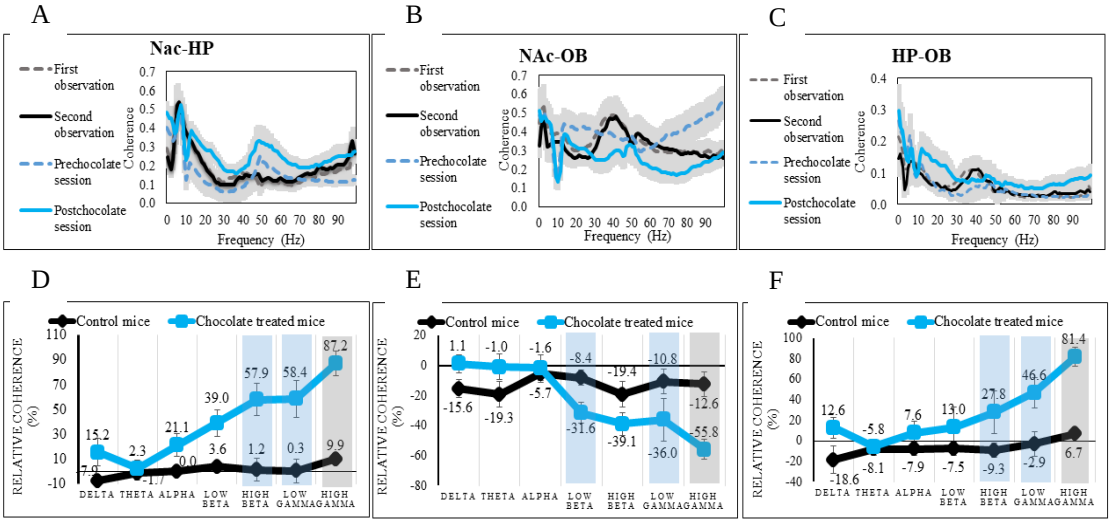

Figure 4.16 Interarial coherence differences in NAc-HP, NAc-OB and HP-OB. Data were averaged and expressed as mean ± S.E.M. The influences of group treatment were determined by using two-way ANOVA followed by Tukey's post hoc test. Blue marks indicates the significant difference at $p<0.05$, and gray marks indicates the significant difference at $p<0.001$

4.4 Discussion and conclusion

This study has pinpointed the hippocampus as the important region of the brain system involved in learning acquisition, storage, and expression of reward memory. It is important to investigate neural network activity for emotion succeeded the concepts of affects, hedonic tones, or emotional feelings. Repeated chocolate consumptions appeared to have rewarding effects correlated with the increase in hippocampal theta-high gamma coupling following chocolate session treated for a week to animals (Fig 4.17). Coherence analysis provided insights over four associated brain regions into the roles of neural process in prediction of reinforcement in habit formation.

Exploration in food zones reflects the feeling of liking or wanting particular foods. In this study, animals were fed with fluid food to induce satiety as confirmed with blood glucose increase that resulted in increased feeling of satiety (Chaput and Tremblay, 2009). Food exploring behavior can be induced by the scent of food no mater they were in satiety state (Todd et al., 2012). In this study, animals also increased time spent at rich chocolate surround and number of locomotor count during exploration after a week session of chocolate even they were satiated. This behavioral pattern did not occur in control mice. Reinforcing property of chocolate was reinstated by the scent priming underlying learning process and associative cues (La Mela et al., 2010). Altogether of behavioral findings, the succession of chocolate or palatable food consumption might have a profound impact on incentive or reward values on foods particularly palatable food. The relationships among intrinsic reward value, identification of stimuli and motivation are mediated by the function of hippocampus (Gilboa et al., 2014).

The responses in the hippocampus to the experience of learning palatable food provided evidence for the functional relevance of synchronization in the network in ingestive experiences and food preference behavior. Previously, neurobiological profiles of hippocampal dysfunctions were reviewed and concluded for the deficits of inhibitory function, Pavlovian learning, stimulus response and working memory mechanism (Douglas, 1967). The role in inhibitory processes of appetitive conditioning via the formation of Pavlovian associations between food and contextual

stimuli in the hippocampus were clearly observed when excitotoxicity hippocampal lesion could produce overeating in animals (Ito et al., 2005). In addition, inactivation of the hippocampus also caused a blockage of place learning and response (Packard and McGaugh, 1996). Hippocampal function in eating behavior was emphasized by the present study as hippocampal LFP oscillations in this study were remarkably sensitive to repeated chocolate consumptions. Increases in power activities of theta and delta oscillation were noticed during post-chocolate session. In particular, low theta and low gamma frequencies appeared to predict movement activity as seen in positive correlation between maximal locomotor speed activity and maximal power frequency parameters.

The increase in high gamma amplitude modulated by theta phase following chocolate sessions might indicate brain mechanism of modulation patterns of reward properties to palatable food preference. Previously, rhymical slow theta activity (RSA; 7-9 Hz) in the hippocampus was not associated with speed of movement (Whishaw and Vanderwolf, 1973). Instead, RSA was related with higher level control of voluntary movement. The ‘on’ state of the brain reported to the increase of theta frequency synchronization in the hippocampus reflects the process of stimulus response (Buzsaki, 2002). The mechanism of gamma oscillation in the hippocampus can be reviewed under key operations of inhibition process on neural groups for separated inputs of memory encoding in which slow gamma range (~40 Hz) entrained from CA3 region and fast gamma (~80 Hz) entrained from entorhinal cortex (Colgin and Moser, 2010). Therefore, gamma oscillation indicated the essentials for the information flow to communicate between brain structures and mediating routs to prevent interference from previous learned association. As well, the cross-frequency coupling between different sub-bands is the general brain mechanism to modulate neuronal assemblies in voluntary behavior (Tort et al., 2008). In human, these oscillations are endowed to cognitive mechanism (Axmacher et al., 2010; Canolty et al., 2006; Mormann et al., 2005).

Although it remains difficult to characterize connectivity of large-scale neural interactions across the entire brain for each specific behavior, this study constitutes the brain connectivity via the pairwise among the HP, NAc, LHa and OB in liking palatable food (chocolate) by using magnitude-coherence analysis. In association to

the roots of reinforcement of chocolate, beta and gamma coherent activities were found to be increased in the pairs of NAc-LHa, NAc-HP, LHa-OB and HP-OB (Fig 4.18). This oscillation was decreased in coherent activity between the pairs of NAc-OB and LHa-HP. Based on the evidences of beta and gamma oscillation related across regions, the degree of coherences of the bands were dependent on intrinsic fluctuation of the synchronization in the brain reported to visual processing, multi-stable perception, associative learning and selective attention (Brovelli et al., 2004; Colgin et al., 2009; Lipsman et al., 2014; Meador et al., 2002; Miltner et al., 1999; Roberts et al., 2013; Sehatpour et al., 2008). These findings allowed for directly imaging the extent of networks in space, time and frequency to manipulate processing inputs from external cues and internal signal sensing to generate and enhance more preference to chocolate.

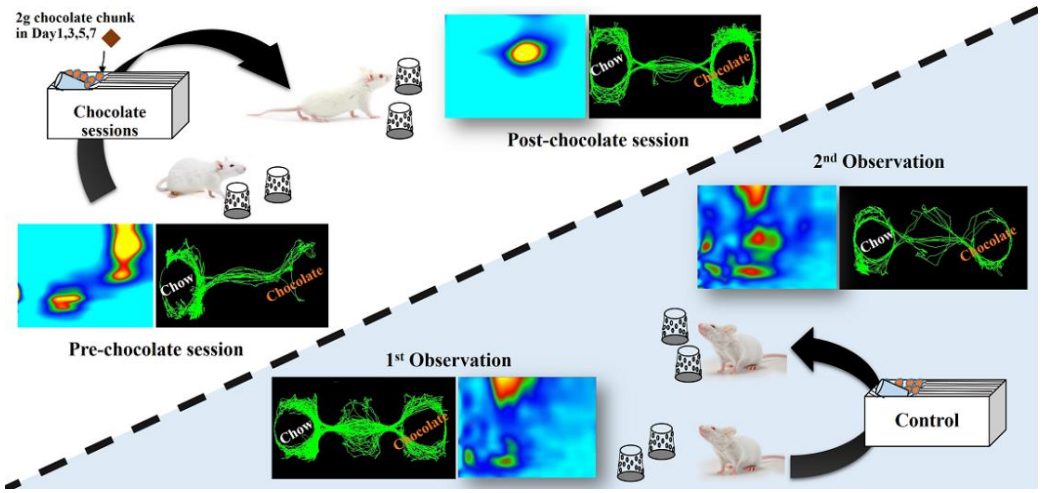

Figure 4.17 Schematic diagram of coupling oscillation and preference-liked behavior

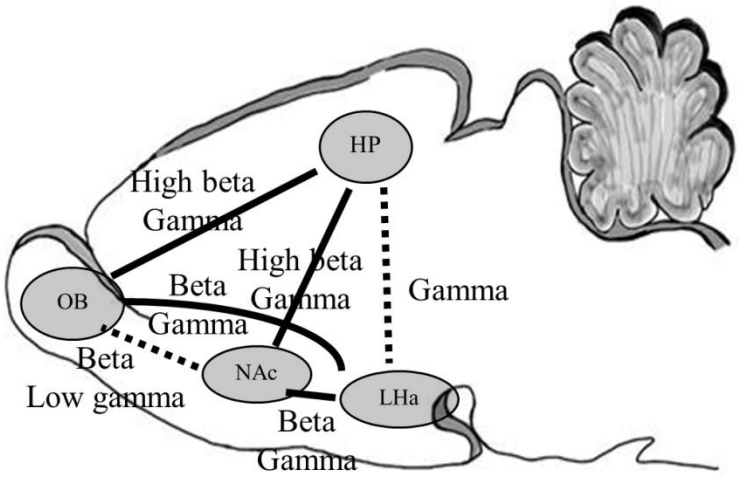

Figure 4.18 Drawing a summary of network brain mechanism induced by a cue of palatable food following a session of repeated chocolate exposures. Solid lines indicate the increased coherence between regions, and dot lines indicate the decreased coherence between regions

CHAPTER 5
CONCLUSION

This study was performed in a major focus to understand how the brain works in neural processes of wanting or liking palatable foods. Better understanding in how brain mechanism of emotional drive to eat might be used to prevent the overeating in the future. Particularly, it was important to employ the biomarkers of neural process that underlie motivation for eating palatable foods and homeostatic hunger from food deprivation. With neural signaling analysis, it was capable to distinguish between neural network activities of hunger and appetite especially for palatable food. The first series of this study was to characterize LFP biomarkers of negative and positive energy status. Ultimately, neural signaling analysis was an ideal methodology to characterize LFP oscillatory pattern that represent brain mechanism of hedonic need for palatable food with frequency modulation in the hippocampus and other related regions. These research purposes were achieved with the experimental design that mice were exposed to choices of normal food (chow) and palatable food (chocolate) following repeated sessions of chocolate learning. The employments of power analysis, spectral coherence analysis and phase-amplitude coupling analysis were necessary to characterize patterns of LFP oscillation specific for hedonic but not homeostatic need.

For increasing beta or gamma powers in hunger period, the activities were increased in the regions of LHa, NAc and HP, but not in the OB. Because there was no scent stimulus exposure during recording, the response in term of mean power activity at frequency ranges was not observed in the OB. The beta and gamma coherence at resting in the context of hunger were increased for LHa-HP and NAc-OB whereas the slow gamma represented the decreased patterns in pair-regions of LHa-NAc and NAc-HP. Because the beta and gamma synchronization across regions have been observed for the cross-link interaction where beta content was linked to the long-range communication and gamma bands were associated to the local neural assembly, the decrease of low gamma coherence activity might suggest the

interference of local synchronized neurons in the neural pathway. On the other hands, the increased beta and gamma coherence indicated the strength of interactions between regions. As observed from the overall findings in this objective, the beta and gamma oscillation might be associated with reinforced properties in the neural network in response to food deprivation. Therefore, LFPs can be used as biomarkers of food hunger in general. It is useful to detect the real aspects of negative energy caused of hunger, and this could help eliminate overeating.

In the study of the palatable food, the results indicated that the preference-like behavior in satiated mice seemed to reflect motivation to seek for liked food. Therefore, the dynamic frequency oscillation in animals following a session of chocolate learning was characterized. The induced patterns of neural processing in the hippocampus and of the NAc, LHa and OB were recognized as highlights of the present study. Following chocolate intake session, theta and gamma coupling also exhibited a higher cross-linking density compared to that of control mice. Additionally, beta and gamma coherence between LHa-NAc, NAc-HP, LHa-OB and HP-OB exhibited the highest strength as well. The decreases of beta and gamma coherence provided a relatively low state of interactions. The large-scale system for four interested sites therefore possesses relatively roughness to path for learning motivational process of palatability required towards the succession of food addition.

Altogether, the LFP analysis is the sensitive technique used for detection of neural activity patterns related with behaviors. The frequency analysis determined via power oscillation activity is recommended as the tool to find the activity in the brain over frequency series. In the case of coherence method applied for inter-regional interaction, it is very much dependent on the degree of the processing in the local and long-range processes. In the view of neural processing across frequency in specification to cognitive mechanism, cross-frequency coupling is highly suggested because the process itself is generally sustained for producing the continuous process in learning mechanism and selective attention. When considering the difference of LFP patterns between homeostatic hunger and palatable food appetite by using coherence analysis of raw LFP signals from the four regions, commutated coherence patterns were observed. Likewise, the increase of LHa-HP and NAc-OB interaction

were accepted to food hunger, and the increase of LHa-NAc, LHa-OB, NAc-HP including HP-OB were downright to exploring behavior of palatable foods.

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APPENDIX

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This is to certify that the research project entitled "EEG biomarkers of neural process related to eating behavior in mice" which was conducted by Asst. Prof. Dr. Ekkasit Kumarnsit , Faculty of Science , Prince of Songkla University, has been approved by The Animal Ethic Committee, Prince of Songkla University.

Kitja Sawangjaroen

Kitja Sawangjaroen, Ph.D.
Chairman,
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ORIGINAL PAPER

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

Nifareeda Samerphob · Dania Cheaha · Surapong Chatpun · Ekkasit Kumarnsit

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 food 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

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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

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Hippocampal CA1 local field potential oscillations induced by olfactory cue of liked food

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ABSTRACT

Eating motivation is induced not only by negative energy balance but also food related cues. However, neural processing for acquisition of learned food preference remains to be established. This study aimed to identify hippocampal neural signaling in response to olfactory cue (chocolate scent) after completion of repetitive chocolate sessions. Male Swiss albino mice implanted with intracranial electrode into the hippocampus were used for local field potential (LFP) recording. Animals were given chocolate sessions (a piece of 2 g chocolate per each mouse to eat on day 1, 3, 5 and 7). Hippocampal CA1 LFP signals and exploratory behavior of animals receiving chocolate scent were analyzed before and after chocolate sessions. The experiment was performed in a place preference-like apparatus with the zones of normal food pellet and chocolate (both kept in a small perforated cup for smell dispersion) at the opposite ends. Following chocolate sessions, time spent in a chocolate zone and CA1 LFP patterns were analyzed in comparison to control levels. Two-way ANOVA revealed significant increase in time spent seeking for chocolate. Frequency analysis of LFP power spectra revealed significant increases in delta and theta powers. Phase-amplitude analysis showed significant increase in maximal modulation index and decrease in frequency for phase of theta-high gamma coupling. Taken together, neural signaling in the hippocampus was sensitive to chocolate olfactory cue that might underlie learning process in response to repeated chocolate consumptions that primed intense food approaching behavior. Ultimately, these LFP patterns might reflect motivation to eat and predict feeding probability.

1. Introduction

Motivation appears to be important in regulation of behavior to satisfy basic requirements for life in particular. It might be discussed as intrinsic and extrinsic types that energize or activate people to do some actions (Ryan & Deci, 2000). These types of motivation are operated through different brain regions. A study using event-related functional magnetic resonance imaging (fMRI) revealed the association between insular cortex activity and engagement decisions weighing the presence of spontaneous self-satisfactions such as interest and enjoyment (Lee, Reeve, Xue, & Xiong, 2012). Various types of behavior are found to be influenced by motivation. Modulation of feeding behaviors by motivation has been discussed extensively probably due to its impact on energy homeostasis. Eating of particular food in human was clearly affected by the hedonic preference and reinforcing motivation (Epstein, Truesdale, Wojcik, Paluch, & Raynor, 2003). This might explain why sometimes eating depends on liking and motivation more than regulation of energy homeostasis. Hedonic preference for food has been investigated in terms of neurotransmitter activity. Previously, increased hedonic values of saccharin and ethanol were blocked by naltrexone, an opioid antagonist (Rodefer, Campbell, Cosgrove, & Carroll, 1999). Additionally, the association between dopaminergic system and hedonic impact of reinforcing value of feeding and drinking has been discussed (Berridge, Venier, & Robinson, 1989). Experimental animals continued to eat in response to the auditory cue linked with food despite being satiated (Reppucci & Petrovich, 2012). This suggests the impact of feeding related condition that can override homeostatic mechanisms. The forebrain network composed of the amygdala, lateral hypothalamus and medial prefrontal cortex has been reported to mediate cue-driven feeding, while a distinct amygdala

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