Local Field Potential Signals Induced by Repeated Exposures and Withdrawal of Morphine in Mice

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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 สัญญาณ local field potential ที่ถูกชักนำโดยการได้รับมอร์ฟีนซ้ำๆ และการถอนมอร์ฟีนในหนูขาวเล็ก

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บทคัดย่อ

โดยทั่วไปยาเสพติดมีคุณสมบัติรีวอร์ดทั้งในมนุษย์และในสัตว์ทดลอง จากการศึกษาที่ผ่านมาพบว่าสมองบริเวณ nucleus accumbens (NAc) มีความสำคัญต่อผลของรีวอร์ด ของยาเสพติด นอกจากนี้ สมองบริเวณ striatum ซึ่งเป็นส่วนหนึ่งของระบบสารสื่อประสาทโดปามีนที่มีหน้าที่เกี่ยวกับการควบคุมการเคลื่อนไหวก็มีสมมติฐานว่ามีบทบาทต่อรีวอร์ดด้วย มีความเชื่อกันว่าคุณสมบัติรีวอร์ดของยาเสพติดทำให้เกิดการใช้ยาเสพติดซ้ำและนำไปสู่การเสพติดยาเสพติด การวิจัยครั้งนี้มีวัตถุประสงค์เพื่อศึกษาการเปลี่ยนแปลงคลื่นศักย์ไฟฟ้าสมอง ในบริเวณ straitum และ NAc ในช่วงที่ได้รับมอร์ฟีนแบบเฉียบพลัน แบบต่อเนื่อง และช่วงถอนมอร์ฟีน การทดลองนี้ใช้หนูสายพันธุ์ ICR เพศผู้ น้ำหนัก 35-40 กรัม ที่ได้รับการผ่าตัดฝัง electrode ในสมองบริเวณ straitum และ NAc จากนั้นสัตว์ทดลองถูกนำมาบันทึกคลื่นไฟฟ้าสมองและพฤติกรรมการเคลื่อนไหว การวิเคราะห์ทางสถิติใช้ one-way ANOVA ตามด้วย Tukey method ผลการทดลองพบว่า มอร์ฟีน (5 และ 15 mg/kg, i.p.) แบบเฉียบพลันมีผลเพิ่ม power ของคลื่นไฟฟ้าสมองของแถบความถี่ low และ high gamma ของทั้ง straitum และ NAc โดยเฉพาะอย่างยิ่งเมื่อได้รับมอร์ฟีนความเข้มข้นสูง อย่างไรก็ตาม power ของคลื่น high gamma ใน striatum มีความสัมพันธ์กับระดับการเคลื่อนไหวที่เพิ่มขึ้น ในขณะที่ power ของคลื่น high gamma ใน NAc ไม่สัมพันธ์กับระดับการเคลื่อนไหว นอกจากนี้ การศึกษาฤทธิ์ของมอร์ฟีนแบบต่อเนื่องและขณะถอนมอร์ฟีนโดยใช้อุปกรณ์ condition place preference (CPP) พบว่า การได้รับมอร์ฟีนแบบต่อเนื่องมีผลลด power ของคลื่นไฟฟ้าสมอง alpha ใน striatum แต่ไม่พบ ใน NAc ส่วนในช่วงถอนมอร์ฟีน พบว่า power ของคลื่น alpha เพิ่มขึ้นใน NAc แต่ไม่พบ ใน striatum โดยสรุปการศึกษานี้แสดงให้เห็นว่า สมองของ straitum และ NAc มีการตอบสนองในการสร้างสัญญาณคลื่นไฟฟ้าสมองต่อการได้รับมอร์ฟีน และแสดงให้เห็นถึงความไวของการเปลี่ยนแปลงคลื่นไฟฟ้าสมองที่อาจสะท้อนถึงกลไกในระบบ รีวอร์ดของยาเสพติด

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Abstract

In general, addictive drugs possess a reward property both in human and animals. Previous reports indicated that the nucleus accumbens (NAc) plays a critical role in a reward action of addictive drugs. Apart from this, the striatum, a brain region known to be involved in motor system, was also hypothesized to have function in reward. It is believed that drug's reward induces drug intake repetition leading to addiction. This study aimed to investigate patterns of change in local field potential (LFP) in the striatum and the NAc in response to acute, repeated and withdrawal of morphine administration. Male albino ICR mice 35-40 g were used for electrode implantation in the striatum and the NAc. Therefore, animals were subjected for recording of LFP and locomotor activity. One-way ANOVA followed with Tukey method was used for statistical analysis. The results showed that acute single dose of morphine (5 and 15 mg/kg, i.p.) significantly produced increases in low and high gamma power in both the striatum and the NAc especially by the high dose. However, high gamma power in the striatum was positively correlated with locomotor activity. No such correlation was seen for the oscillation in the NAc. In addition, the effects of repeated administration and withdrawal of morphine in the conditioned place preference (CPP) also showed the decrease in alpha power in the striatum but not in the NAc. During morphine withdrawal period, significant increases in alpha power were found in the NAc but not in the striatum. Taken together, the present findings demonstrated that the striatum and the NAc produce some changes in LFPs in response to morphine administration. These data also indicated the sensitivity of oscillatory patterns that might reflect the underlying mechanism of addictive drugs in the reward system.

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Chayaporn Raekkhamnuan

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

CONTENTS **Page**

TABLE OF CONTENTS (continued)

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

LIST OF ABBREVIATIONS AND SYMBOLS

μ	Mu-opioid receptors
δ	Delta-opioid receptors
k	Kappa-opioid receptors
%	Percentage
°C	Degree Celsius
6-OHDA	6-hydroxydopamine
ANOVA	Analysis of variance
cAMP	Cyclic adenosine 5'-monophosphate
CeA	Central amygdala
CNS	Central nervous system
CPP	Condition place preference
CR	Conditioned response
CREB	cAMP response element– binding protein
CS	Conditioned stimuli
D1R-KO	Dopamine one receptor knockout
D2R-KO	Dopamine two receptor knockout
DAT	Dopamine transporter
EEG	Electroencephalography
FFT	Fast Fourier Transform
g	Gram
GABA	Gamma-Aminobutyric acid
hr (s)	Hour (s)
Hz	Hertz
ICR	Imprinting Control Region
i.m.	Intramuscular
i.p.	Intraperitoneal
kHz	Kilohertz
LC	Locus coeruleus

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

LFP	Local field potential
MFB	Medial forebrain bundle
mg/kg	Milligram per kilogram
NAc	Nucleus accumbens
P	P-Value
PSD	Power spectral density
PSU	Prince of Songkla University
S.E.M	Standard Error of Mean
SN	Substantia nigra
SNc	Substantia nigra pars compacta
UCR	Unconditioned response
UCS	Unconditioned stimulus
VTA	Ventral tegmental area

CHAPTER 1

INTRODUCTION

1.1 Background and rationale

Drug abuse is a major public health problem that impacts society on multiple levels in Thailand and worldwide. For Thailand, the numbers of drug convict are progressively increasing every year. Considerably, every community is affected by drug abuse and addiction, even in family. All drug abuse hurts the body and the brain. Some drugs can change the mood, attention and decision making. However, addiction effects overcome by making them feel good leading to obsessive and repeated use. The addiction effects are typically not produced at the first time to begin using drugs. Because drugs abuse is naturally rewarding, they become addicted to drugs only after they use it repeatedly. In addition, the appearance of withdrawal symptoms presents when they do not use drugs.

Since it was established that drugs abused by humans are rewarding (i.e., give an interoceptive pleasurable effect) for humans and for animals, a great deal of research has been devoted to clarify the biological mechanism of drug abuse. However, several findings found that mesolimbic and mesocortical dopamine pathways (dopamine cells in the ventral tegmental area (VTA) projecting to the nucleus accumbens (NAc) and the frontal cortex respectively) are important for the rewarding effects of drugs abuse (Fibiger et al., 1987). Moreover, the other dopamine pathways investigated the nigrostriatal dopamine system (dopamine cells in substantia nigra (SN) projecting into the dorsal striatum). In addition, neurosciencetists purpose that drugs abuse might activate brain reward systems. By repeated administration, the biological structures of reward brain areas could be affected to develop addiction (Koob and Le Moal, 1997).

Morphine is an opioid drug, and the main psychoactive chemical in opium. For almost as long, it has been known that the therapeutic use of opiates for analgesia. It acts directly on the central nervous system (CNS) to decrease the feeling

of pain. However, morphine also has a high potential for addiction and abuse. Abrupt cessation or reduction of the dose after long term use appears to induce withdrawal. Recently, morphine has been reported the effect in two terminal dopaminergic areas, the NAc and the dorsal striatum (Wise, 2009). However, it is necessary to find the question by using scientific method.

This study was performed in a mice model. Animals were rendered depending on brain area that received dopaminergic inputs from the VTA and the SN during morphine acute, administration and withdrawal. Electrical brain signal has been considered to represent the activity of the brain. Thus, this study used local field potential (LFP) to investigate NAc and dorsal striatum brain area during morphine acute, administration and withdrawal.

1.2 Review of literature

1.2.1 Reward

Research has identified a biological mechanism mediating behavior commonly associated with pleasure. Pleasurable state describes a feeling of happiness or satisfaction resulting from experiences that one enjoys. This mechanism is called "reward" (Esch and Stefano, 2004). A reward is a stimulation given to a human or some other animals to change behaviors of subjects for example, when eating of a salted peanut triggers the motivation for more salted peanuts. Rewards are short-lived and not stored in long-term memory, but they can influence the probability and vigor of the next response such as addictive drugs (Wise, 2009). In neuroscience term, reward has been focused on functions of neural structures that are critically involved in mediating the effects of reinforcement and regulation of behaviors that are associated with pleasurable, desirable, approachable, or intrinsically positive stimuli. In neurobiology, pleasure is a competence or function of the reward and motivation circuitries that are imbedded in the CNS. Anatomically, these reward pathways are particularly linked to the brain's limbic system.

Drugs of abuse are very powerful reinforcers, they motivate high rates of operant responding. In the studies of neuropharmacology and neuroanatomy of brain reward, 3 major brain systems appear to be involved in drug reward which includes dopaminergic, opioidergic and GABAergic pathways. It has been clear that these evidences confirm the involvement of a midbrain-forebrain circuit particularly with its focus in the NAc in reward function (Koob, 1992b).

1.2.2 Dopamine

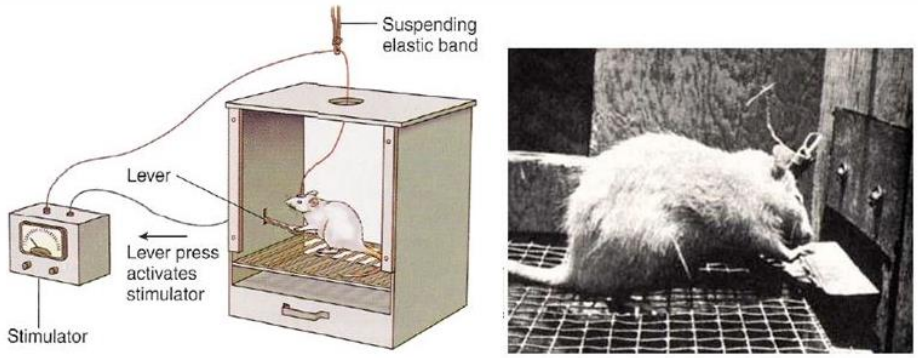


Fig. 1.1 Self-stimulation experiment of Olds and Milner: rat reaches eagerly for a switch to activate stimulation (Modified from Olds and Milner, 1954).

Dopamine plays several important roles in the brain and body. In the brain, dopamine functions as a neurotransmitter. Dopamine neurotransmitter is a key player in pleasure and reward physiology. There is much evidence for a strong relationship. The brain's reward and motivation circuitry were discovered in the 1954 (Esch and Stefano, 2004). In experiments by Olds and Milner (1954), specialized centers for reward functions have been demonstrated. In their studies, electrical stimulation of specific brain sites was found to be highly rewarding in the sense that rats responded operantly to electrical stimulation. A neurotransmitter system that is particularly sensitive to electrical self-stimulation is dopamine (Spanagel and Weiss, 1999). Several research supported dopamine's association with mechanisms involved in addiction, pleasure and reward. Opiate antagonists are able to block the dopamine agonistic activation of the brain reward system (Kornetsky, 2004). Thereby, dopamine and opiates/opioid may be interconnected in the drug-reward (Thompson and Kristal, 1996; Kornetsky, 2004). In addition, some research showed that low dopamine may further be associated with continuous drug consumption, since repeated use of cocaine or morphine, for example, may deplete dopamine from mesolimbic dopamine system and reward circuitry (Nestler, 2001). These dopamine depletions may be responsible for changes of normal rewards and related to the intense craving associated with withdrawal in human drug addicts (Nestler, 2005). Thus, dopamine neurotransmitter represents important roles in reward and addiction.

1.2.3 Dopamine pathway

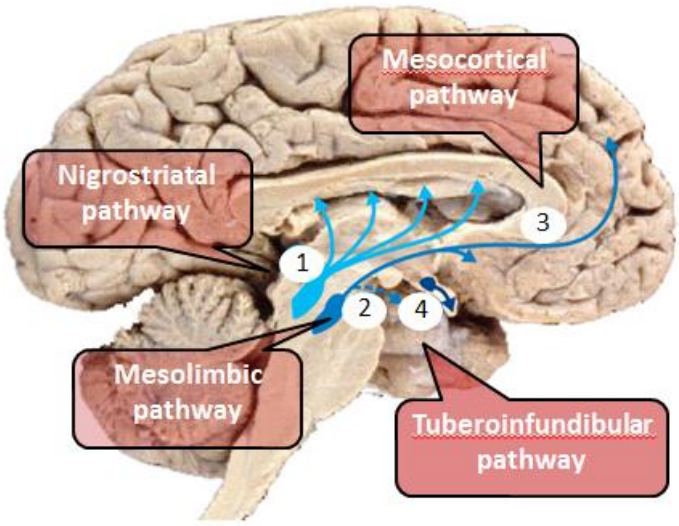


Fig. 1.2 Four major dopamine pathways in the CNS.

Dopamine was synthesized in dopaminergic neurons and released to other parts of the brain via axons that run the entire length through the brain pathway. The cell bodies of most of these neurons lie in two contiguous regions of the midbrain: the substantia nigra pars compacta (SNc) and VTA. This small number of midbrain dopamine neurons innervates extensive terminal fields within the forebrain. Neurons from the SNc densely innervate the dorsal striatum where they play a critical role in learning and executing motor programs. Neurons from the VTA innervate the NAc, the olfactory bulb, the amygdala, the hippocampus, the orbital and the medial prefrontal cortex and the cingulate cortex. VTA dopamine neurons play a critical role in motivation, reward, attention, and multiple forms of memory. Altogether, the main dopamine pathways can be divided to four majors pathways.

1.2.2.1 Nigrostriatal pathway

The nigrostriatal pathway or nigrostriatal system projects dopamine from the SN to the dorsal striatum. It is involved in movement control in a system called the basal ganglia motor loop.

1.2.2.2 Mesolimbic pathway

The mesolimbic pathway or mesolimbic system projects dopamine from the VTA to NAc. It is significantly involved with reward and pleasure. Hence, it has been of great interest in studies of motivation and addition, where it has been linked with central mechanisms of alcohol, nicotine and cocaine.

1.2.2.3 Mesocortical pathway

The mesocortical pathway or mesocortical system projects dopamine from the VTA to the frontal lobe of the pre-frontal cortex. The mesocortical pathway is believed to play a role in motivational and emotional responses.

1.2.2.4 Tuberoinfundibular pathway

The tuberoinfundibular pathway projects dopamine from the hypothalamus to the pituitary gland. Dopamine released here regulates secretion of prolactin from the anterior pituitary gland (Volkow et al., 2011).

1.2.4 Reward and mesolimbic dopamine

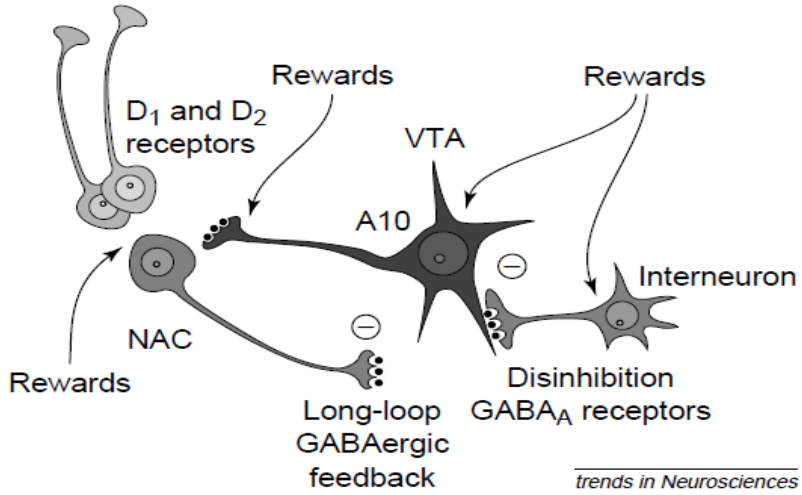


Fig. 1.3 The mesolimbic dopamine pathway arised from dopaminergic cell bodies in VTA. These dopaminergic axons project to the NAc, also called the ventral striatum (Spanagel and Weiss, 1999; Carr and Sesack, 2000).

The medial forebrain bundle (MFB) originates from the ventral midbrain to the basal forebrain. It has long been associated with reward function. The MFB consists of a population of myelinated fibres that project to the VTA. A significant proportion of VTA neurones are dopaminergic that project to the NAc. This connection is known as the mesolimbic pathway. Electrical stimulation through electrodes implanted along this bundle was considered to be pleasurable or rewarding because animals appeared to perform certain task repeatedly such as pressing a bar to trigger the stimulation (self-stimulation) (Olds and Milner, 1954; Olds, 1962). The studies of Heath in humans demonstrated that subjects would similarly self-administer electrical stimuli to specific “pleasure” areas of the brain (Heath, 1963; Heath, 1972). Subsequently, the brain structures, neuronal pathways, and relevant neurotransmitters implicated with reward and reinforcement were further refined (us reviewed by Adinoff, 2004). Taken together, the experience of reward is at least accompanied by activation of the mesolimbic dopaminergic pathway. Moreover, natural rewards, such as food or sex

as well as most substances that are abused by humans also were also hypothesized to increase extracellular concentrations of the mesolimbic dopamine.

The stimulants such as cocaine have been demonstrated to increase the synaptic release of dopamine in the mesolimbic dopamine system (Koob, 1992a). Cocaine is thought to act mainly by blocking the presynaptic dopamine transporter (DAT) involved in reuptake (Ritz et al., 1987). Additionally, there are non-stimulant drugs that indirectly interact with the mesolimbic pathways through a variety of receptor systems. These compounds share the common pharmacologic property in stimulating mesolimbic dopamine primarily in the NAc. These non-stimulant drugs bind with G protein-coupled receptors of opiates such as heroin or morphine (agonists at opioid receptors, activating dopamine release via VTA GABAergic disinhibition) (Johnson and North, 1992). Alternatively, they can also bind with ligand-gated ion channel receptors such as in the case of alcohol or nicotine products like cigarettes (agonists for nicotinic cholinergic receptors).

Some studies of drug reward confirmed the importance of the mesolimbic pathway such as in the anatomical study by Bozarth and Wise (1981). Morphine was administered into the VTA region of rats trained to self-administer morphine into the VTA. Following the administration of the opioid antagonist, no signs of physical dependence were produced. This study demonstrated biological processes involved in reward that also supported the role of the VTA in drug reward (Pettit and Justice, 1991). Furthermore, preclinical studies have directly considered the effects of increasing or decreasing dopamine in the NAc upon drug self-administration. In the study by Pettit and Justice (1991) using an in vivo microdialysis technique, a significant correlation between the amount of cocaine self-administered by rodents and the extracellular dopamine released in the NAc was found. Conversely, the self-administration was decreased following administration of dopamine antagonists (Bergman et al., 1990). These studies suggested the role of mesolimbic pathway in drug reward.

1.2.5 Roles of nigrostriatal dopamine pathway in reward and addiction.

Dopamine circuitry has traditionally been studied in terms of reward and addiction. First, many studies focused on the role of the mesolimbic pathway which has dopamine cells in the VTA projecting to the NAc. Second, others focused on motor related nigrostriatal dopamine system with dopamine cells in the SN projecting into the dorsal striatum. Interestingly, consistent evidence appeared to suggest that both systems participate in reward function and addiction (Wise, 2009). Anatomically, there is no clear boundaries that completely separate these two midbrain dopamine systems (Wang and Morales, 2008). In addition, tracing study demonstrated that SN and VTA dopamine cells have overlapping, not distinct, projection fields. Ultimately, brain stimulation has been applied to study the mapping of reward-related circuitry in the brain by using direct electrical stimulation to certain brain regions. The population of midbrain dopamine neurons was seen as a final common pathway for the rewarding effect of MFB stimulation (Wise, 2009). However reward sites are found both in the SN and the VTA (Crow, 1972). Movable electrode mapping studies also indicated reward related brain sites within these areas of the dopaminergic cell body regions, as called the SN and the VTA (Wise, 1981). Previously, the SNc was demonstrated to produce dopamine to innervate the dorsal striatum, the brain area involved in motor and reward processes (Delgado, 2007). Taken together, the nigrostriatal dopamine system has been found to possess similar properties to that of the mesolimbic dopamine system in participation of reward function and addiction (Wise, 2009). Thus, the nigrostriatal dopamine system, at least in part, has been found to be involved in reward and addiction in parallel to that of the mesolimbic dopamine system.

1.2.6. Systems involved in opiate self-administration

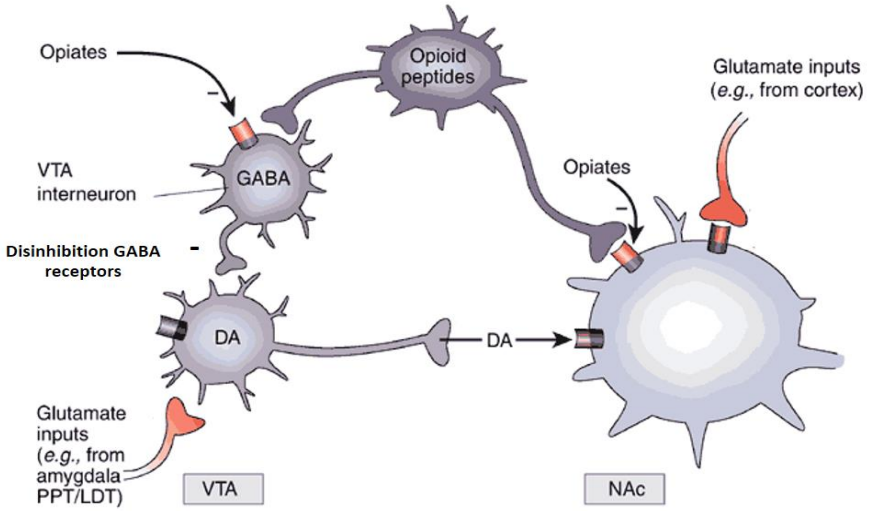


Fig. 1.4 The neural mechanisms of opiate on mesolimbic pathway (Volkow et al., 2011).

Opiate drugs exert their pharmacological actions through 3 opioid receptor subtypes, mu (μ), delta (δ) and kappa (k)-opioid receptors mimicking the actions of endogenous opioid peptides including endorphins, endomorphines, enkephalins and dynorphins. The μ-opioid receptors subtype is critical for the rewarding effects of heroin and morphine (De Vries and Shippenberg, 2002). *In vivo* microdialysis has been used to examine the effects of selective μ, δ and k-opioid receptors on dopamine release in the NAc of anesthetized rats and demonstrated positive reinforcers and significant increase in extracellular dopamine through activation of μ -pioid receptors (Spanagel et al., 1990). Blockade of μ-opioid receptors but no other opioid receptors abolished the conditioned preferences previously associated with opiate administration (Spanagel et al., 1990; Matthes et al., 1996). Basically, neuronal circuit studies of opiate addiction have focused on the mesolimbic dopamine system (De Vries and Shippenberg, 2002). This structure is characterized by a high density of μ-opioid receptors (Mansour et al., 1995).

Opiate-induced activation of VTA dopamine cells could be mediated indirectly (Gysling and Wang, 1983; Johnson and North, 1992), via an activation of

μ-opiate receptors located on GABAergic midbrain interneurons that negatively regulate dopamine cell firing (Di Chiara and Imperato, 1988; Johnson and North, 1992). Activation of these inhibitory $G_{\alpha i}$-coupled μ-opiate receptors reduces the GABAergic tone onto midbrain dopamine neurons, thereby increasing their firing rate and the amount of dopamine released in the NAc (Koob, 1992b; Hnasko et al., 2005).

1.2.7. Local field potential (LFP)

The LFP recoding technique is widely used for both clinical diagnosis and research studies. Basically, the electrical signal generated by neuronal network in the brain. The signal recordings from microelectrodes penetrated directly through the brain represent specific electrical activity from specific neuronal networks. The LFP signal from the recording is a result of the summation of potentials derived from the mixture of extracellular currents generated by populations of neurons.

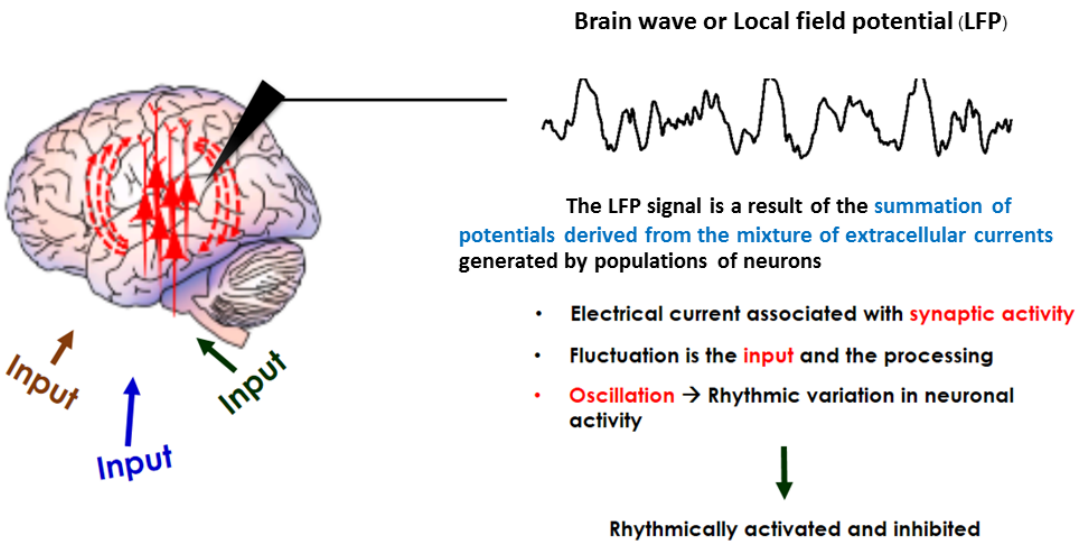


Fig. 1.5 Local field potential (LFP) signal reflects the electrical currents associated with synaptic activity in local population of neurons around the electrode.

LFP signal reflects the electrical currents associated with synaptic activity in local population of neurons around the electrode. It is also thought to

represent the summation of excitatory and inhibitory dendritic signals. The oscillation of LFP is resulted from the inputs rhythmically activated and inhibited in the neural network. In principle, LFP signals typically consist of complex waveforms that are composed of rhythms with different frequency mixtures. Neuronal oscillation in specific neuronal network can be analyzed by various measures. Among various power spectral analyses, individual frequency bands associated with relatively specific functional role were focused.

Basically, LFP signals are non-linear and nonstationary in nature. Thus, it is difficult to get useful information from LFP signal directly in the time domain by visual observation. The frequency representation of LFP signals is the most powerful and standard one. Frequency analysis of LFP wave first developed by Jean Baptiste Fourier, a French mathematician and physicist is also known as Fourier transformation or power spectral analysis. It is applied in signal processing including that for biological signals such as LFP. Furthermore, the Fourier Transform is computationally attractive since it can be calculated by using an extremely efficient algorithm called the Fast Fourier Transform (FFT).

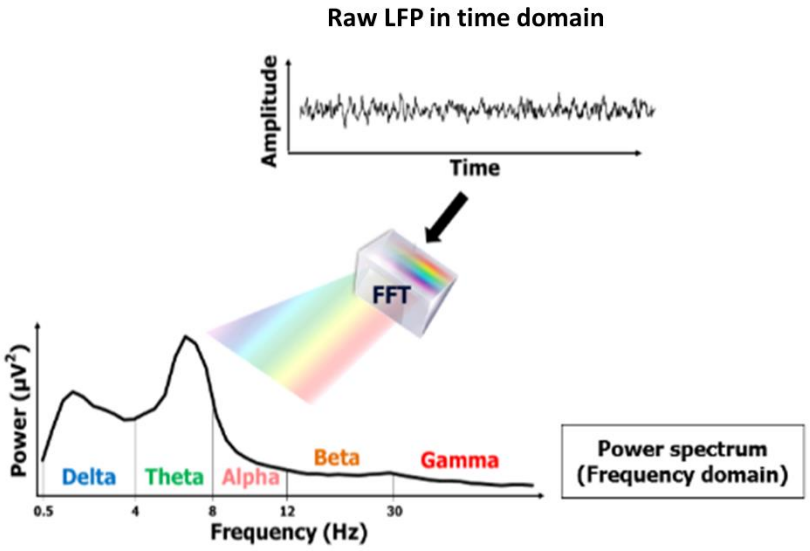


Fig. 1.6 The fast fourier transform (FFT) process representation. It transforms the discrete LFP signal from time domain into frequency domain as shown.

The FFT transforms the discrete LFP signal in time domain into the frequency domain (Figure 1.6). The frequency of LFP signal mostly depends on network components for example the types of neuron, receptors or neurotransmitters. The LFP signal is traditionally divided into broad frequency band such as delta, theta, alpha, beta and gamma frequency ranges. The division is not a one standard across the literatures but mainly arises from characteristic of oscillation behaviors observed at the specific frequencies. Variations of LFP frequency correlates with the activity of neural network. Different neural networks generate different LFP frequencies which are associated with the network function.

1.3 Objectives

In this study, signal processing tool was employed to distinguish special neural signaling of acute, continuous administration and withdrawal of morphine. This was in purpose for better understanding of neural circuit processing of drug abuse.

The major aim was to investigate the LFP signals recorded in morphine sensitive brain regions (NAc and striatum) during acute, chronic and withdrawal morphine period in order to search for LFP biomarkers.

The specific objectives are shown as followings.

1 Changes in LFP oscillation induced by acute morphine administration. (CHAPTER3)

2 Patterns of LFP oscillation in response to repeated morphine administration and during morphine withdrawal period in CPP paradigm. (CHAPTER4)

CHAPTER 2

RESEARCH METHODOLOGY

1. Animals

Male Swiss albino ICR mice with body weight ranging from 35-40 g were used for the whole experiment of this study. They were provided by the Southern Laboratory Animal Facility of Prince of Songkla University (PSU), Songkhla, Thailand. They were housed in standard environmental conditions with temperature at 23-25 °C, 50-55% humidity and 12:12 hr. light: dark cycle. Standard commercial food pellets and distilled water were supplied *ad libitum*. The experimental protocols for care and use of experimental animals described in the present study were approved and guided by the Animals Ethical Committee of PSU. The project license number is MOE 0521.11/840

2. Chemical agents and drugs

2.1 Surgery

1. Acrylic resin (Unifasttrad, Tokyo, Japan)
2. Lidocaine sulphate (Locana, L.B.S. Laboratory Ltd., Part., Thailand)
3. Ketamine (Calypsol, Gedeon Richter Ltd., Hungary)
4. Xylavet (Xylavet, Thai Maji Pharmaceutical Co., Ltd., Thailand)
5. Calypsol (Gedeon Richter Ltd., Hungary)
6. Ampicillin (General Drug House Co., Ltd., Thailand)

2.2 Drug treatment

1. Morphine sulfate ($C_{17}H_{19}NO_3$) (Zentiva, a.s., Hlohovec, Slovakia)

3. Equipments

1. Animal surgical apparatus sets
2. Stereotaxic apparatus (Narishige Scientific Instrument Lab., Setagayaku, Tokyo, Japan)
3. Electric micro motor dental hand piece (drill) (Marathon, Daegu, Korea)
4. Stainless steel screws
5. Silver wire electrodes (A-M system, Sequim, WA, USA)
6. 8-channel sockets
7. Bio Amp cable (AD Instrument Pty Ltd, Australia)
8. EEG Bio Amp cable (AD Instrument Pty Ltd, Sydney, Australia)
9. PC computer
10. PowerLab (AD Instrument Pty Ltd, Sydney, Australia)
11. Recording chamber
12. Webcam video camera
13. Condition place preference apparatus

4. Methods

4.1 Local field potential

4.1.1 Animal surgery for intracranial electrode implantation

For surgical procedure, animals underwent stereotaxic implantation of electrodes for LFP recording. Surgery was performed under ketamine/xylazine (150/15 mg/kg) given by intramuscular (i.m.) injection. Anesthetic depth was monitored and tested using a gentle toe-pinch withdraw reflex. The surgery was started when the proper depth of anesthesia was reached. This was judged with the loss of withdrawal reflex. This reflex indicates whether the animal responds to pain or not. Anesthetic depth was also intensively monitored during the surgery process. Any reaction of too light anesthesia from the animals was responded with additional anesthetic top-ups. Fur of animal's head was shaved off with scissors. Then, each animal was placed onto the stereotaxic apparatus and mice's head was clamped with the apparatus. Ear bar of the apparatus was inserted into each side of ear canals and checked for proper tightness between ear bars and ear canals by gentle pressing and shaking animal's head. Proper fixation was confirmed when mice's head could not wobble. Mice's incisor was attached to incisor bar and animal's tongue was pulled out of the mouth to prevent chocking during surgery (Fig. 2.1). A nose clamp was held to cover mice's nose properly. Both incisor bar and nose clamp were used to secure animal's head with apparatus and the position of head on stereotaxic apparatus was fixed in the standard position for the electrode implantation on the skull. Briefly, the scalp was swabbed with 70% alcohol and followed by betadine. After lidocaine sulphate (20 mg/ml) was injected subcutaneously, a skin midline incision long from the lambda to just in-between the eye was made on the scalp. The skin was pinched off by using sterile hemostats and therefore the incision was kept open. All connective tissues were removed and the exposed skull surface was dried off.

The LFP electrodes were implanted on the skull at the following positions (Fig. 2.2)

1. Striatum with co-ordinates: 1.5 mm lateral to the left, 1 mm anterior and 3.5 mm dorsal-ventral from the bregma.

2. Nucleus accumbens (NAc) with co-ordinates: 1.3 mm lateral to the left, 0.7 mm anterior and 4.7 mm dorsal-ventral from the bregma.

3. Midline cerebellum with co-ordinates: 6.5 mm posterior to bregma was used as reference and ground electrode.

Additional holes were drilled for stainless steel anchor screws (Fig. 2.3). All the electrodes (Fig. 2.4) were linked to a female connector fixed to the skull by dental cement. After surgery, animals were placed in a clean cage with a heating pad and monitored until ambulatory behavior was observed. Antibiotic (100 mg/kg ampicillin) was applied intramuscularly for 3 days to prevent infection. They were allowed to fully recover for at least 7 – 10 days before the start of the experiment.

To validate, histological localization of the electrode and specific LFPs patterns are vital. First, coordinates for brain area were marked by following the previous studies for placing the electrodes in the striatum (Pan et al., 2010) and the NAc (Olive et al., 2000). After that, mouse brain atlas was used to confirm the coordinates. Addition, LFP patterns of the striatum and the NAc in this study were checked with the published LFP patterns seen in the previous studies (Gada et al., 2013; Dimpfel and Schombert, 2015).

Fig. 2.1 Preparation of mice for surgery in a stereotaxic apparatus. The apparatus mainly includes head holders and a calibrated mechanism. Mice's skull was held in a standard position. The assigned co-ordinates for the striatum, NAc and cerebellum were accurately marked for electrode placement.

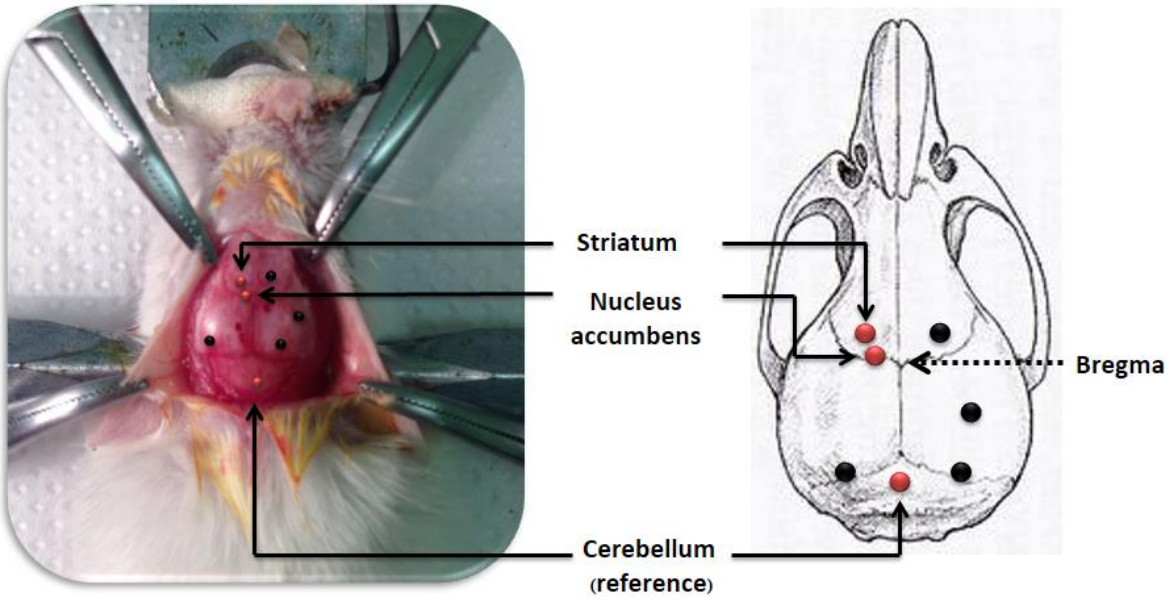


Fig. 2.2 Coordination for electrodes (red points) and stainless steel screw anchors (black points).

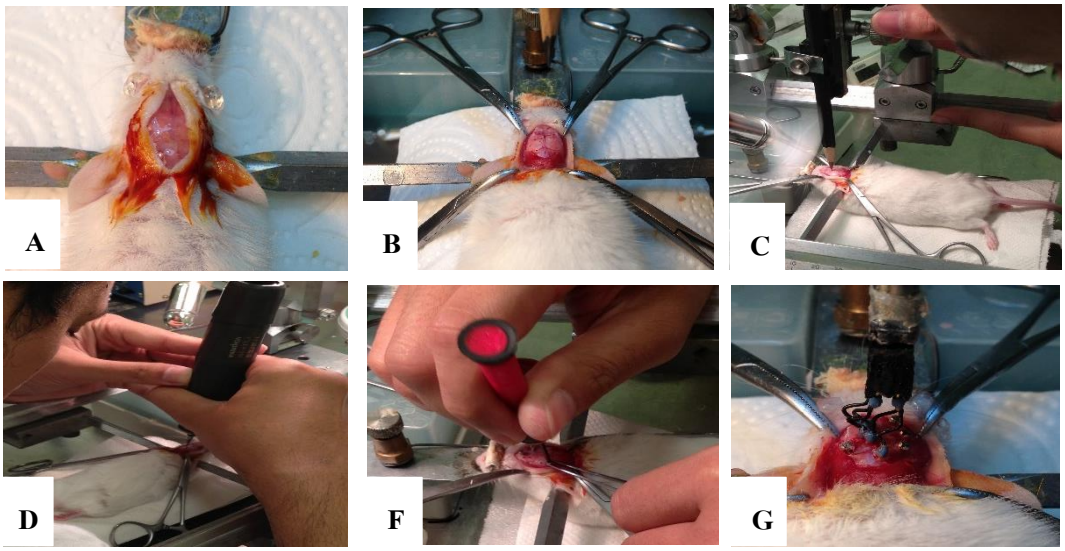


Fig. 2.3 The processes of electrode implantation and binding of electrode connectors. The processes included scalp incision (A), the incision was kept open by using hemostats (B), marking electrode locations (C), the skull was drilled in the marked position (D), electrodes and stainless steel anchor screws were implanted in marked position (F and G).

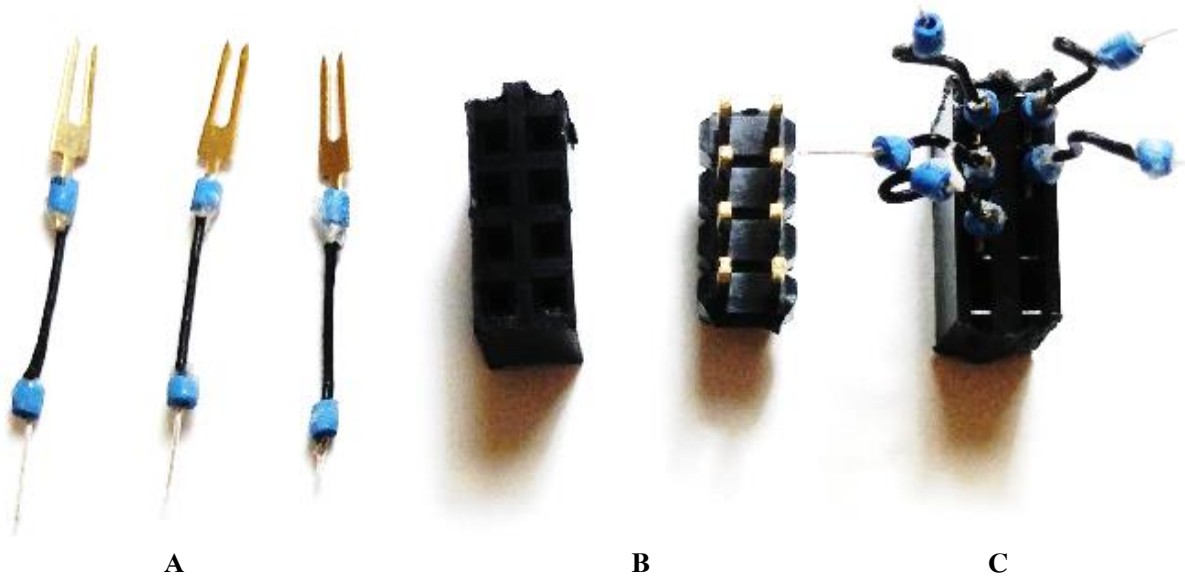


Fig. 2.4 Accessories for electrode implantation. There are wire electrodes for the striatum, nuclus accumbens (NAc) and cerebellum (ground and reference) (A) and male and female connectors (B). All electrodes were bound together in a socket (C).

4.1.2. Signal recording and data acquisition

An LFP recording unit was composed of hardware and apparatus including chamber, recording cable, BioAmp set, Power Lab and a computer (Fig. 2.5). A webcam was mounted on the stand for top view recording of locomotor activity. For recording protocol, LFP electrodes on mice's skull were connected with recording cables via a 6-channel socket (Fig. 2.6). After that, animals were individually put into the recording chamber. Then, recording cables were connected to LFP recording unit via a BioAmp set. At this stage, a BioAmp set was connected to recording electrodes from the striatum and NAc through the 6 channel socket. During recording, signals were processed via the BioAmp and PowerLab box for signal amplification, filtering and digitization. The setting values of parameters for recording were precisely controlled through the Chart software. LFP signals were amplified with low-pass 200 Hz, high-pass 1 Hz and digitized at 2 kHz by a PowerLab 16/35 system (AD Instruments, Castle Hill. NSW, Australia) with 16-bit A/D. Data were stored in a computer through the LabChart 7 program software. 50 Hz notch filtering was applied to remove the noise from power line artifacts. All LFP signals were processed through 1–200 Hz band-pass digital filter (raw filtered signal). By the way, during recording, signal was illustrated and real-time monitor by using spectrogram (Fig 2.7).

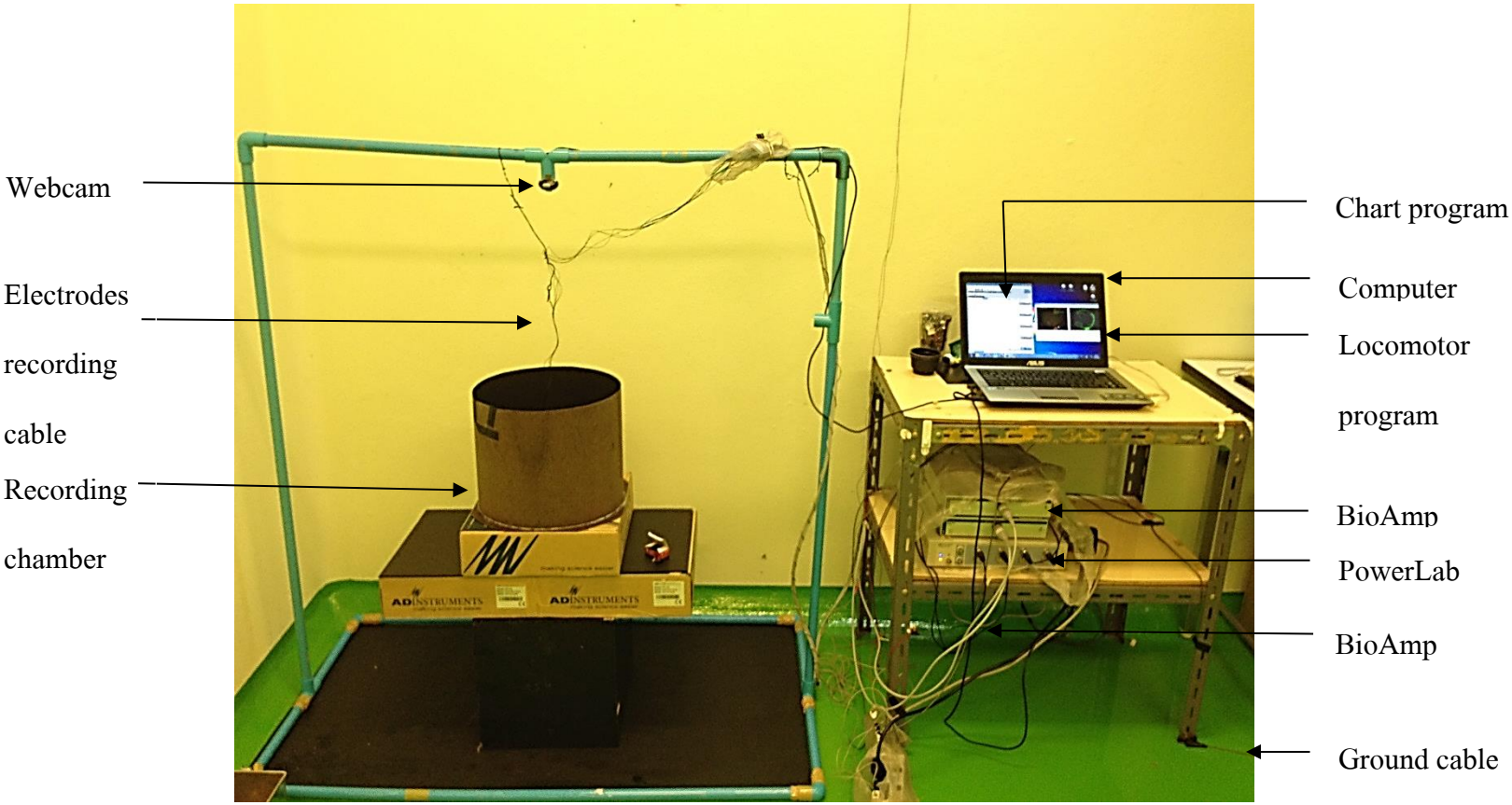


Fig. 2.5 The whole setup for local field potential (LFP) recording. The recording system was mainly composed of a recording chamber, recording cables, BioAmp sets, a PowerLab box, a ground cable and a computer. A webcam was mounted on the stand for top view recording of locomotor activity**.**

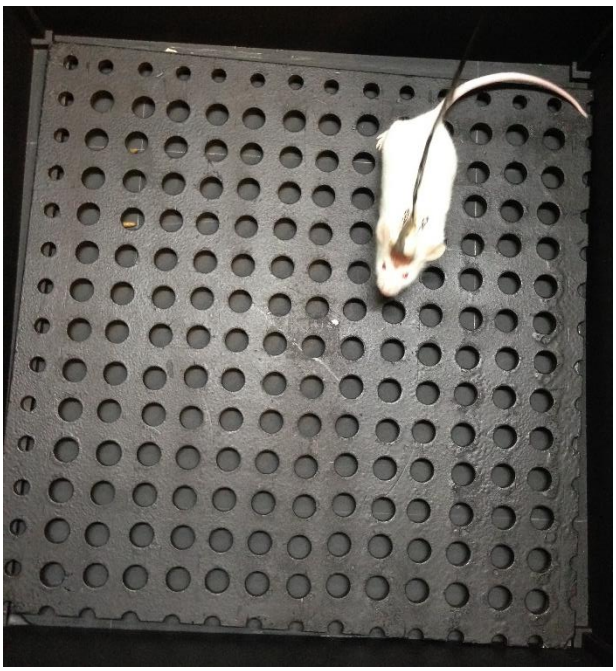


Fig. 2.6 The connection of local field potential (LFP) electrodes from animal's skull with recording system. The LFP electrodes were connected with the recording cable through a socket. An individual animal was put in the recording chamber.

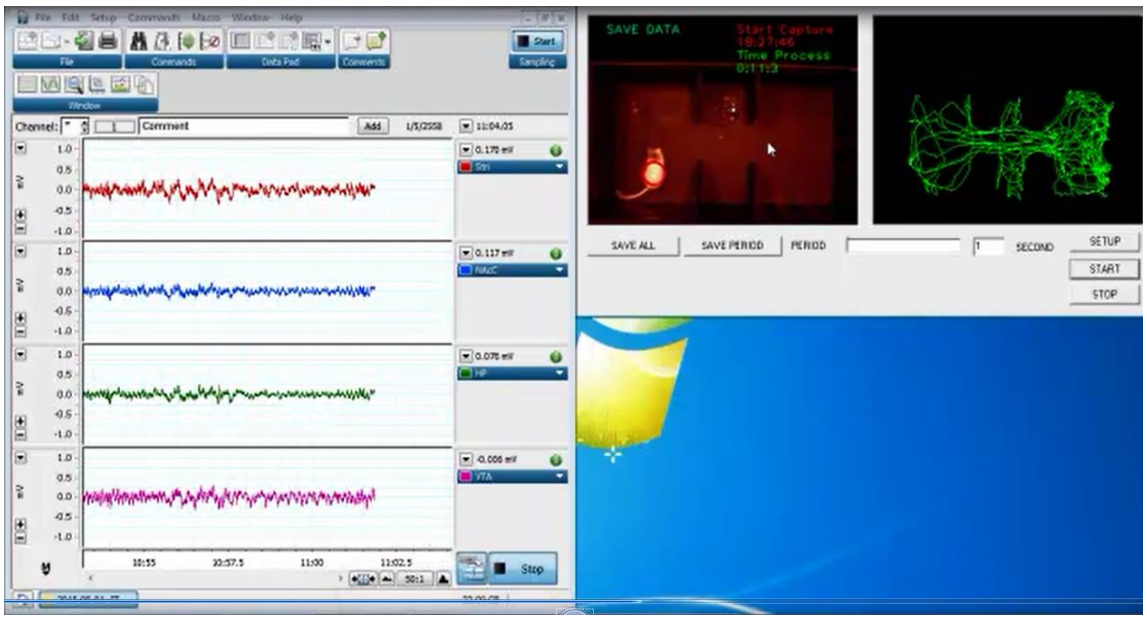


Fig. 2.7 The monitoring system during signal recording

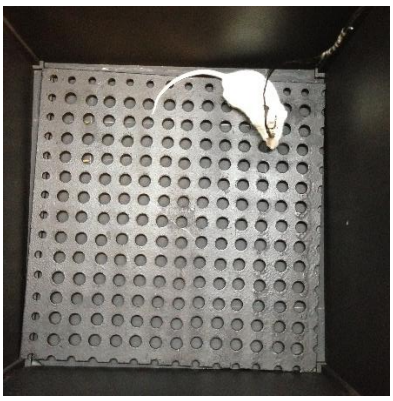
4.1.3 Offline signal analysis

All data of LFP signal were recorded while animal exploring in the chamber. All LFP signals were amplified, then filtered with a low-pass 200 Hz, high-pass 1 Hz and digitized at 2 kHz by a PowerLab 16/35 system (AD Instruments, Castle Hill. NSW, Australia) with 16-bit A/D. Data were stored in a computer through the LabChart 7 program software. 50 Hz notch filtering was applied to remove the noise from power line artifacts. To avoid 50 Hz noise, the signal from 45-60 Hz was sometimes excluded from the further analysis.

For spectral power analysis, power spectral density (PSD) was generated by LabChart 7 software using Hanning window cosine with 50% window overlapping and 0.976 Hz frequency resolution and the data were shown in power spectra and power density ($\mu V^2/Hz$). All LFPsignals were processed through 1–200 Hz band-pass digital filter (raw filtered signal). Then, the power spectra in selected time period was calculated and displayed as percent total power of LFP activity (Fig. 2.8). Percent total power values were calculated by using the following equation.

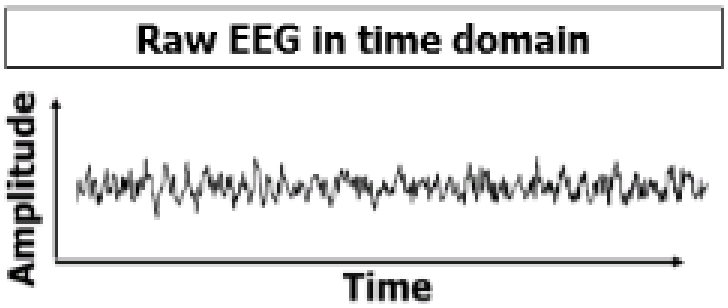
$$\text{Percent total power} = \frac{(\text{Power density of treatment} \times 100)}{\text{Summation of power density of treatment}}$$

The broad frequency band was divided into 7 frequency bands: Delta (1-4 Hz), Theta (4-8 Hz), Alpha (9.7-12 Hz), Beta1 (13.6-18 Hz), Beta2 (19.5-29.3 Hz), Low gamma (30.3-44.9 Hz), High gamma (60.5-95.7 Hz) (Fig. 2.9). Therefore, powers of discrete band were analyzed independently from each other.



Analogue signals from electrodes

Amplified, filtered and digitized



Raw filtered signal

Fast Fourier transform (FFT) algorithm (Hanning window cosine transform)

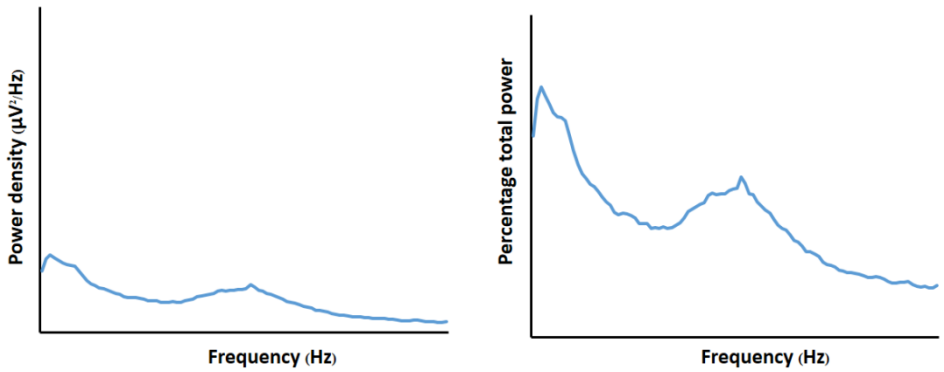


Fig. 2.8 The data processing from signal recording, filtration and until illustration of power spectrum.

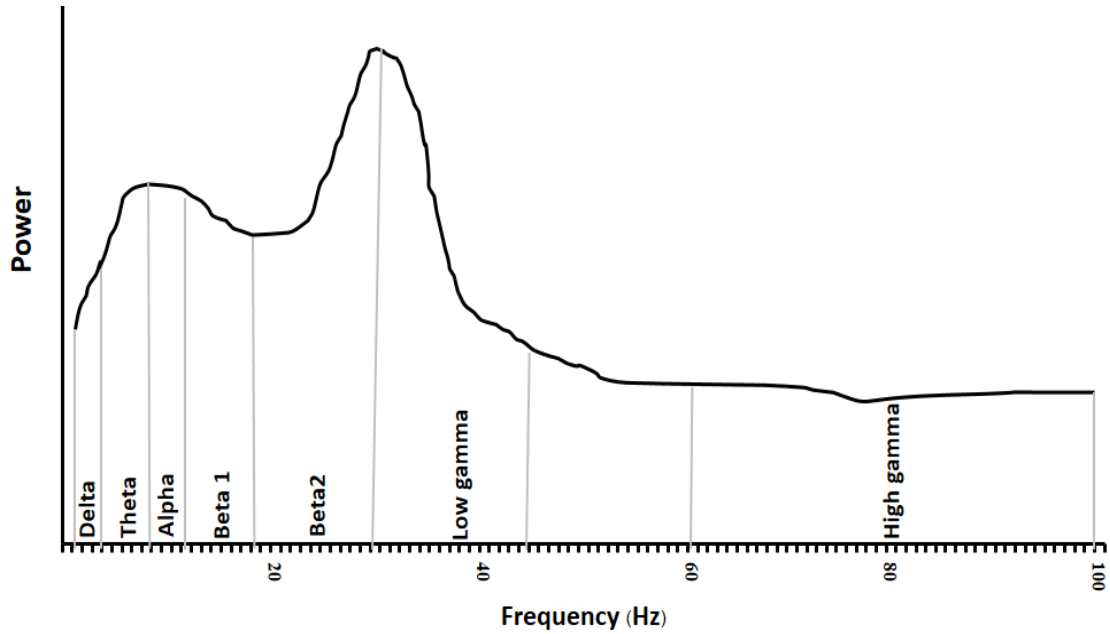


Fig 2.9 Division of spectral power analysis of 1-100 Hz range into individual power of 7 frequency bands. Local field potential (LFP) power of specific frequency bands from Delta (1-4 Hz), Theta (4-8 Hz), Alpha (9.7-12 Hz), Beta1 (13.6-18 Hz), Beta2 (19.5-29.3 Hz), Low gamma (30.3-44.9 Hz), High gamma (60.5-95.7 Hz) range are illustrated.

4.2 Locomotor activity measurement

During LFP recording, spontaneous motor activity was simultaneously recorded by using a webcam from top view position. Animal images were continuously transferred to a computer for data processing. In brief, the computer software (visual C^{++}) was used for analysis of animal movement base on the detection of contrast between animal body (white) and chamber background (black). The center of animal body was tracked (Fig. 2.10). Length and dimension of signal image were validated by using the reference of scale bar. The translocation of animal was detected with the sensitively at 2 mm threshold. One count was considered from 1 period of continuous translocation and 1 stop. Fragments of distance of all translocation during testing period were summed for total distance made by each animal. Levels of locomotor activity were expressed as mean ± Standard Error of Mean (S.E.M.) of cumulative locomotor count and summed distance.

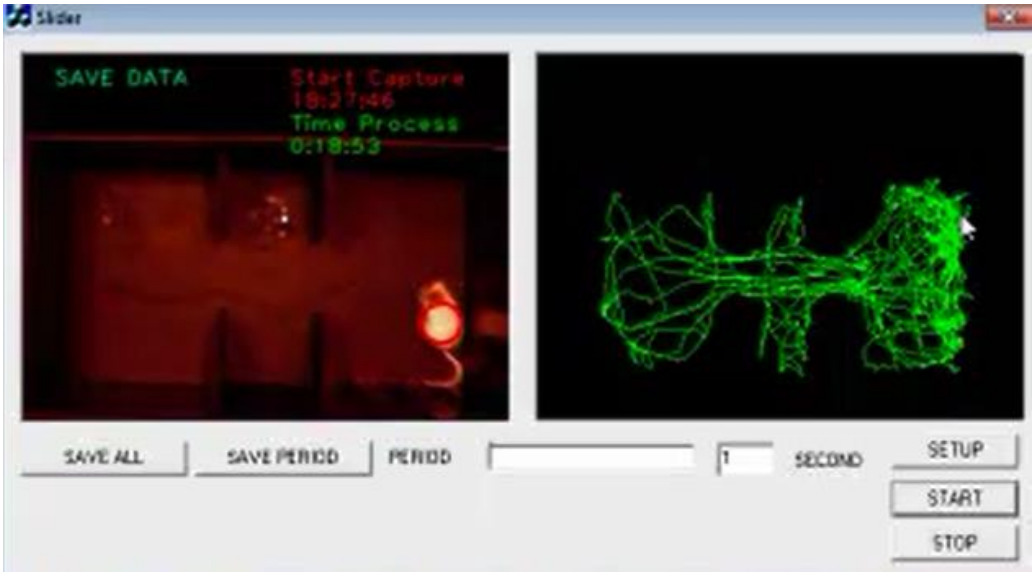


Fig. 2.10 A frame of an image transferred from a webcam to a computer for locomotor activity analysis. A red circle mark represents the whole body and indicates the location of the animal. Patterns of translocation of red circle were analyzed and calculated by using computer software developed in the laboratory.

4.3 Statistical analysis

All data were averaged and expressed as mean ± S.E.M. One- and two-way analysis of variance (ANOVA) were used to determine the influence of one and two factors, respectively. Multiple comparisons were made using Tukey's *post hoc* test to indicate specific points of significance. In addition, linear regression analyses between percentage total power and locomotor speed were also analyzed. Levels of significance were set at $p < 0.05$.

4.4 Experimental design

The experiments were divided into 2 main parts and shown in 2 chapters as followings.

Chapter 3: Changes in LFP oscillation induced by acute morphine administration.

Chapter 4: Patterns of LFP oscillation in response to repeated morphine administration and during morphine withdrawal period in CPP paradigm.

CHAPTER 3

CHANGES IN LOCAL FIELD POTENTIAL OSCILLATION INDUCED BY ACUTE MORPHINE ADMINISTRATION

3.1 Introduction

Drugs abuse are naturally rewarding which is the reason why they are self-administered by laboratory animals or consumed by humans (Volkow et al., 2011). In 1954, the brain was demonstrated to have specialized centers for reward functions (Olds and Milner, 1954). The confirmation was made when these brain regions were electrically stimulated and highly rewarding response was obviously produced. In term of mechanisms, the midbrain dopaminergic system was particularly sensitive to electrical brain self-stimulation as seen in the operant conditioning method used to determine rewarding effects (Spanagel and Weiss, 1999). Dopamine is a neurotransmitter important for the rewarding effects of drugs abuse. The dopamine circuits have been extensively studied as neuronal networks of reward and addiction. Most of previous studies focused on the role of the mesolimbic dopamine pathways. These pathways consist of dopamine cells in the VTA projecting to the NAc. Moreover, the other dopamine system has been studied in Parkinson's disease topics that investigate the nigrostriatal dopamine system with dopamine cells in SN projecting into the dorsal striatum. These are two main separate dopamine systems with different brain areas.

Currently, a new challenge has been focused on the hypothesis whether both systems participate in reward function and addiction (Wise, 2009). Anatomically, there is no clear boundaries that completely separate these two midbrain dopamine systems (Wang and Morales, 2008). In addition, tracing study demonstrated that the SN and VTA dopamine cells have overlapping, not distinct, projection fields. Ultimately, brain stimulation has been applied to study the mapping of reward-related circuitry in the brain by using direct electrical stimulation to certain brain regions. The population of midbrain dopamine neurons was seen as a final

common pathway for the rewarding effect of MFB stimulation (Wise, 2009). However reward sites are found both in the SN and the VTA (Crow, 1972). Movable electrode mapping studies also indicated reward related brain sites within the areas of the dopaminergic cell body regions of the SN and the VTA (Wise, 1981). Previously, the SNc was demonstrated to produce dopamine to innervate the dorsal striatum, the brain area involved in motor and reward processes (Delgado, 2007). Taken together, the nigrostriatal dopamine system has been found to possess similar properties to that of the mesolimbic dopamine system in participation of reward function and addiction (Wise, 2009).

This study aimed to investigate LFP signal of the dorsal striatum and the NAc, the brain area that receive dopaminergic inputs from the SN and VTA, respectively, during morphine administration.

3.2 The experimental procedure for recording of spontaneous LFP signal and locomotor activity during morphine administration

After animals surgery for electrode implantation, they were allowed to fully recover for at least 7 – 10 days. After that the experiment started. Before LFP recording in response to acute morphine administration, the animals were habituated with the recording condition in a chamber for 4 hrs per day for 3 consecutive days. Then, baseline recording for one hour was required before intraperitoneal injection of either saline or morphine (5 or 15 mg/kg). Post-drug recording was performed for 3 hrs following the injection.

During LFP recording, spontaneous motor activity was simultaneously recorded. Locomotor activity of animals was recorded by using a video camera mounted on the top of the recording chamber. The recording method and analysis of locomotor speed were done as previously described (Chapter2).

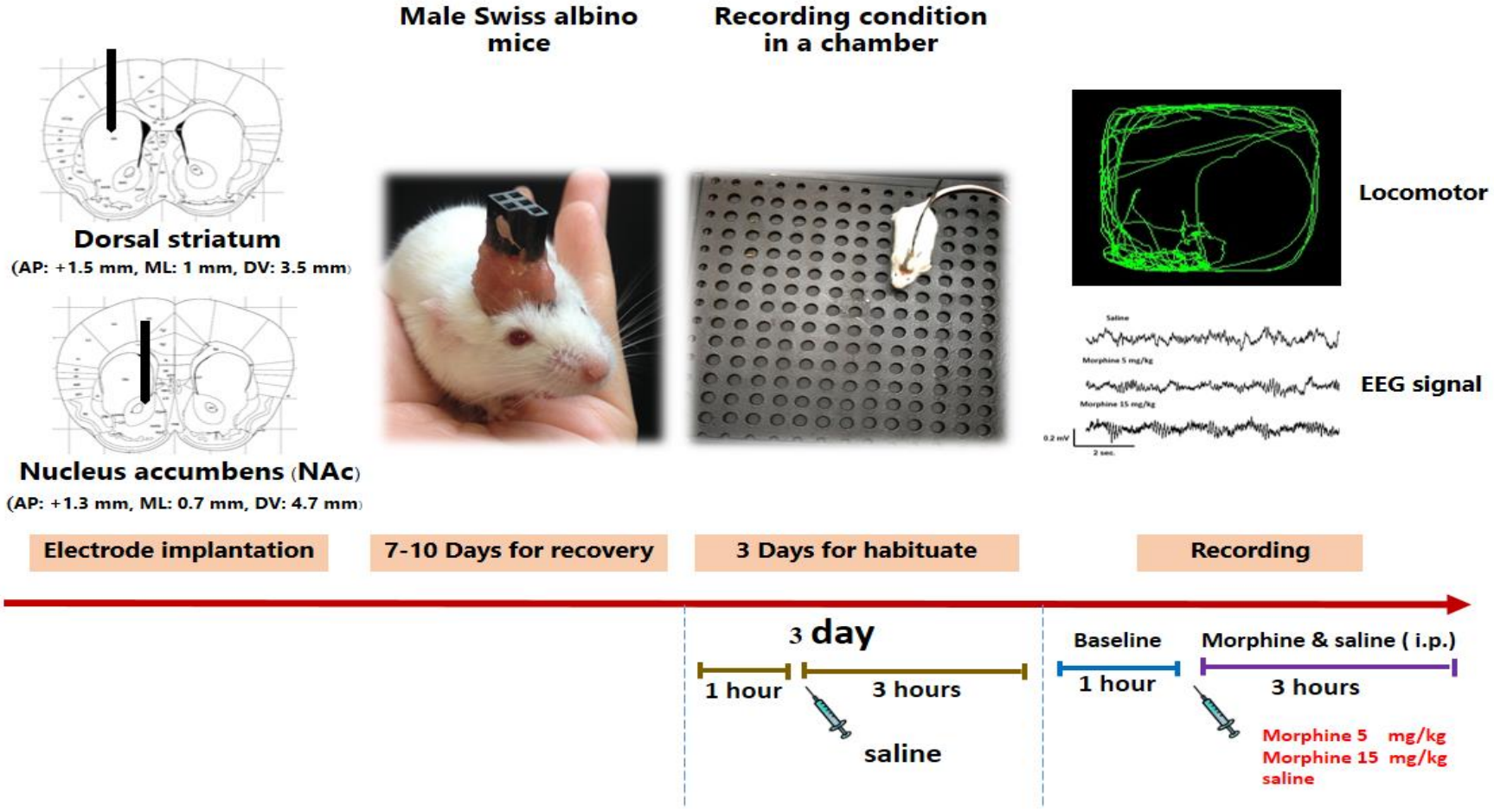


Figure 3.1 Experimental procedure for spontaneous local field potential (LFP) signal and locomotor activity during morphine administration.

3.3 Statistical analysis

All data were averaged and expressed as mean ± S.E.M. Differences between the saline and morphine (5 mg/kg or 15 mg/kg) were analyzed by using one-way analysis of variance (ANOVA) followed by multiple comparisons using Tukey's *post hoc* test to indicate specific points of significance. In addition, linear regression analyses between LFP power and locomotor speed were also analyzed. Levels of significance were set at $p < 0.05$.

3.4 Results

3.4.1 Effects of acute morphine administration on locomotor activity

During LFP recording, spontaneous motor activity was simultaneously recorded by using a webcam from top view. For the effects of morphine administration on locomotor activity the results showed that morphine dose dependently increased averaged speed and averaged max speed in comparison to that of saline control group (Fig. 3.2A and 3.2B). One-way ANOVA revealed that locomotor speed [F (2, 24) = 11.522; $p < 0.001$] and averaged max speed [F (2, 24) = 10.638; $p < 0.001$] were significantly increased in 15 mg/kg morphine group. No significant change was observed in 5 mg/kg morphine group for both locomotor parameters.

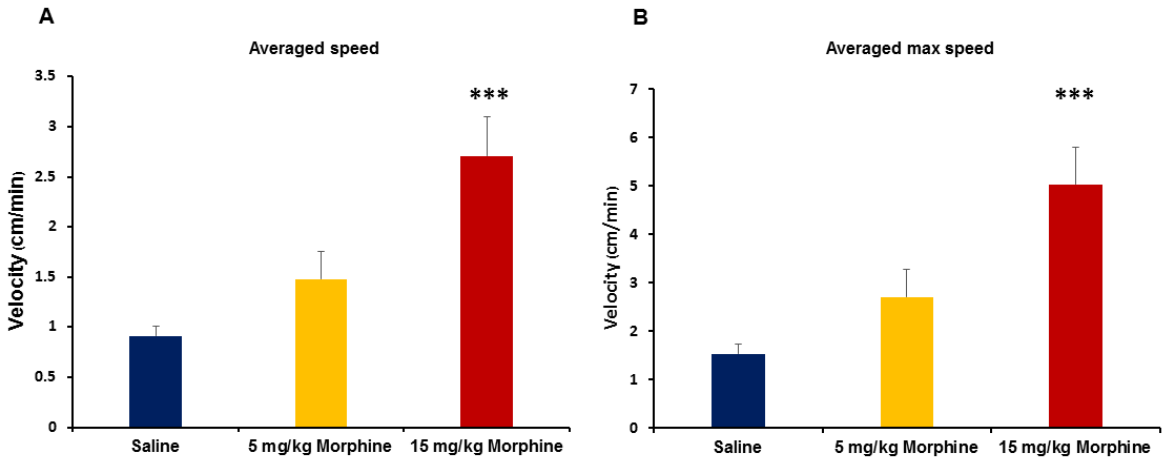


Figure 3.2 Morphine-induced averaged speed and averaged max speed. (A) Averaged speed and (B) Averaged max speed during morphine administration expressed as mean ± S.E.M. Data were analyzed using one-way ANOVA followed by Tukey's *post hoc* test. ***: $p < 0.001$ compared with the saline control group.

3.4.2 Changes in LFP in the striatum following acute morphine administration

Following the administration of saline or morphine (5 and 15 mg/kg), LFP signals from individual mice were continuously recorded for 3 hrs. Obviously, morphine treatment (15 mg/kg) appeared to specifically increase power in a range of

low (30.3-44.9 Hz) and high (60.5-95.7 Hz) gamma oscillation (Fig 3.3A). Representative raw LFP tracings of saline, 5 and 15 mg/kg morphine groups were shown (Fig. 3.3B). By visual inspection, relatively equal slow wave activities of striatum LFP were seen among groups. However, the slow oscillations appeared to be superimposed with fast wave activity in morphine groups particularly at a 15 mg/kg dose.

3.4.2.1 Time-course effects of acute morphine administration on low and high gamma power in the striatum

Frequency analysis of raw LFP during a period of 25-35 min was conducted for percent total power in a broad frequency range from 1 to 100 Hz (Fig. 3.4A). Statistical analysis also confirmed that significant increase in low gamma (30-44.9 Hz) power [$F(2,16)= 5.426$; $P= 0.022$] was seen in the group of high dose of morphine (Fig. 3.4B). No significant difference was produced by 5 mg/kg morphine and frequency analysis of raw LFP during a period of 100-120 min was conducted for percent total power (Fig. 3.5A). Statistical analysis also confirmed that significant increase in high gamma (60.5-95.7 Hz) power [$F(2,16)= 5.864$; $P= 0.010$] was seen in the group of 15 mg/kg morphine (Fig. 3.5B). No significant difference was produced by 5 mg/kg morphine.

Therefore, the effects of morphine treatment on low gamma oscillation in the striatum were particularly analyzed in time domain (Fig. 3.6). Data were converted to percent total power and analyzed every 5 mins. Differences in percent total power of low gamma frequency compared to control levels were determined by using one-way ANOVA followed by Tukey's *post hoc* test. It was found that 15 mg/kg morphine began to produce significant increases from the 15^{th} until 65^{th} min (15 min [$F(2,16)= 4.486$; $P= 0.048$], 20 min [$F(2,16)= 10.533$; $P= 0.003$], 25 min [$F(2,16)= 4.529$; $P= 0.026$], 30 min [$F(2,16)= 8.255$; $P= 0.005$], 40 min [$F(2,16)= 8.459$; $P= 0.003$], 45 min [$F(2,16)= 10.351$; $P= 0.001$], 50 min [$F(2,16)= 5.481$; $P= 0.023$], 55 min [$F(2,16)= 5.856$; $P= 0.017$], 60 min [$F(2,16)= 5.024$; $P= 0.044$] and 65 min [$F(2,16)= 4.098$; $P= 0.039$]). Peak effect was observed during the 30^{th} min. No

significant change in percent total power of low gamma was induced by 5 mg/kg morphine.

Additionaly, effects of morphine treatment on high gamma oscillation in the striatum were particularly analyzed in time domain (Fig. 3.7). Differences in percent total power of high gamma frequency compared to control levels were determined by using one-way ANOVA followed by Tukey's *post hoc* test. It was found that 15 mg/kg morphine began to produce significant increases from the 95^{th} until 140^{th} min (95 min [$F(2,16)= 6.188$; $P= 0.009$], 100 min [$F(2,16)= 4.842$; $P= 0.016$], 105 min [$F(2,16)= 5.215$; $P= 0.014$], 110 min [$F(2,16)= 4.720$; $P= 0.019$], 115 min [$F(2,16)= 4.753$; $P= 0.018$], 120 min [$F(2,16)= 4.204$; $P= 0.036$], 125 min [$F(2,16)= 5.613$; $P= 0.013$], 130 min [$F(2,16)= 3.905$; $P= 0.048$] and 140 min [$F(2,16)= 4.081$; $P= 0.029$]. No significant change in percent total power of high gamma was induced by 5 mg/kg morphine.

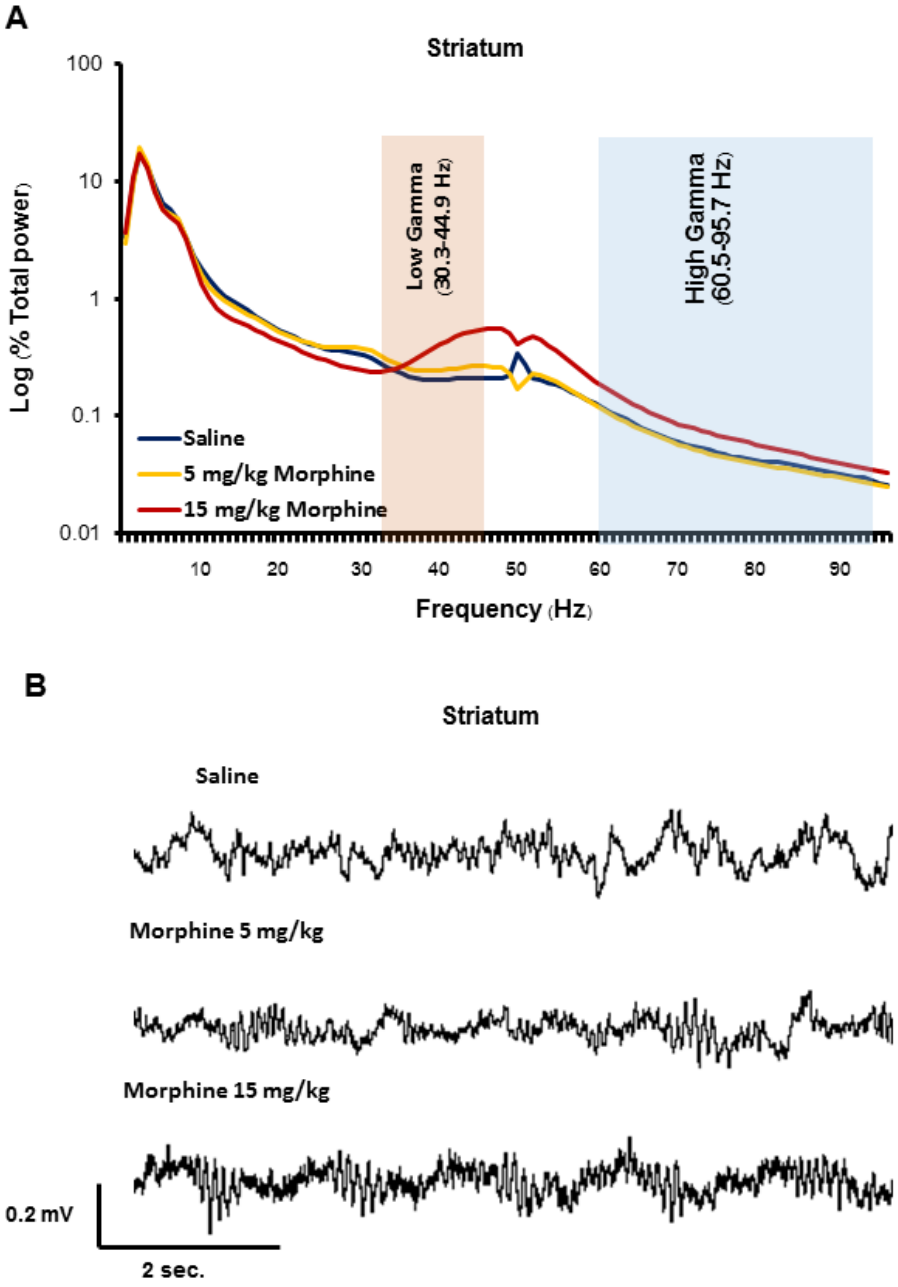


Figure 3.3 Local field potential (LFP) analysis of striatum following acute morphine administration. (A) Power spectrums of striatum LFP are expressed in frequency domain. (B) Raw striatum LFP signals recorded from representative mice that received saline, 5 mg/kg morphine and 15 mg/kg morphine were displayed in time-domain.

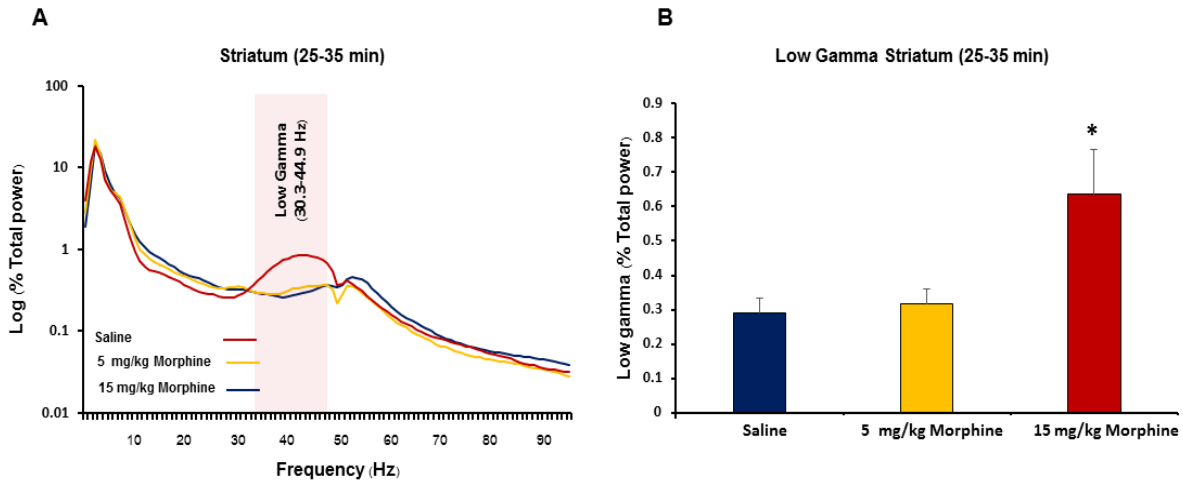


Figure 3.4 Frequency analyses following acute morphine administration. (A) Power spectrums of striatum local field potential (LFP) 25-35 min are expressed in frequency domain. (B) Averaged percent total power of low gamma range are expressed as mean ± S.E.M. * $p < 0.05$ compared with the saline control group (one-way ANOVA followed by Tukey's *post hoc* test).

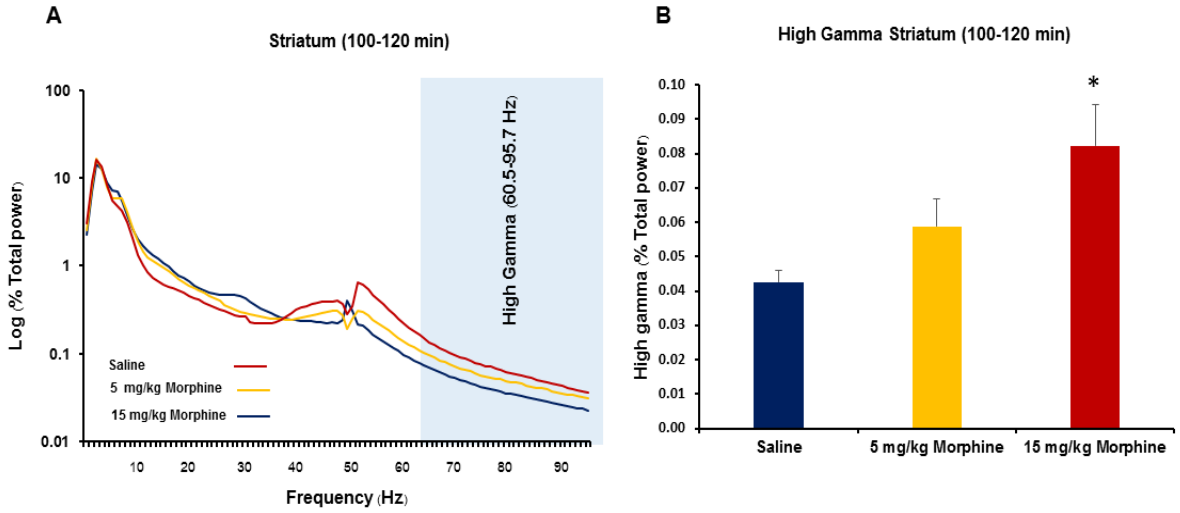


Figure 3.5 Frequency analyses following acute morphine administration. (A) Power spectrums of striatum local field potential (LFP) 100-120 min are expressed in frequency domain. (B) Averaged percent total power of high gamma range are expressed as mean ± S.E.M. * $p < 0.05$ compared with the saline control group (one-way ANOVA followed by Tukey's *post hoc* test).

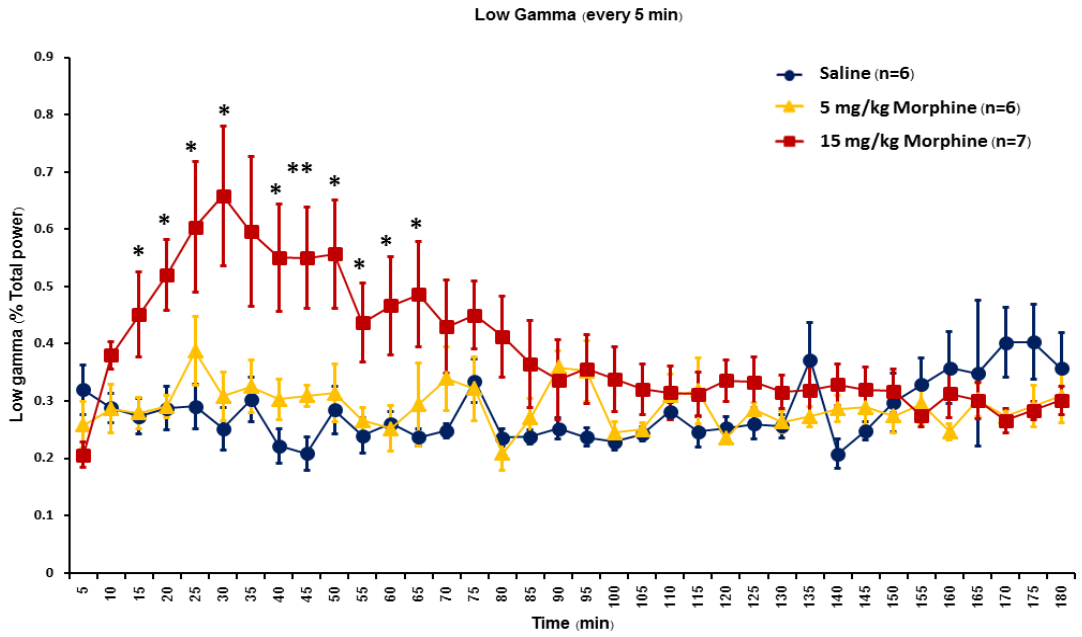


Figure 3.6 The average percent total power of low gamma range were analyzed every 5 min period after injection of morphine (5 and 15 mg/kg) or saline. Data were compared with that of saline control group using one-way ANOVA followed by Tukey's *post hoc* test. *, **: $p < 0.05$ and $p < 0.01$, respectively

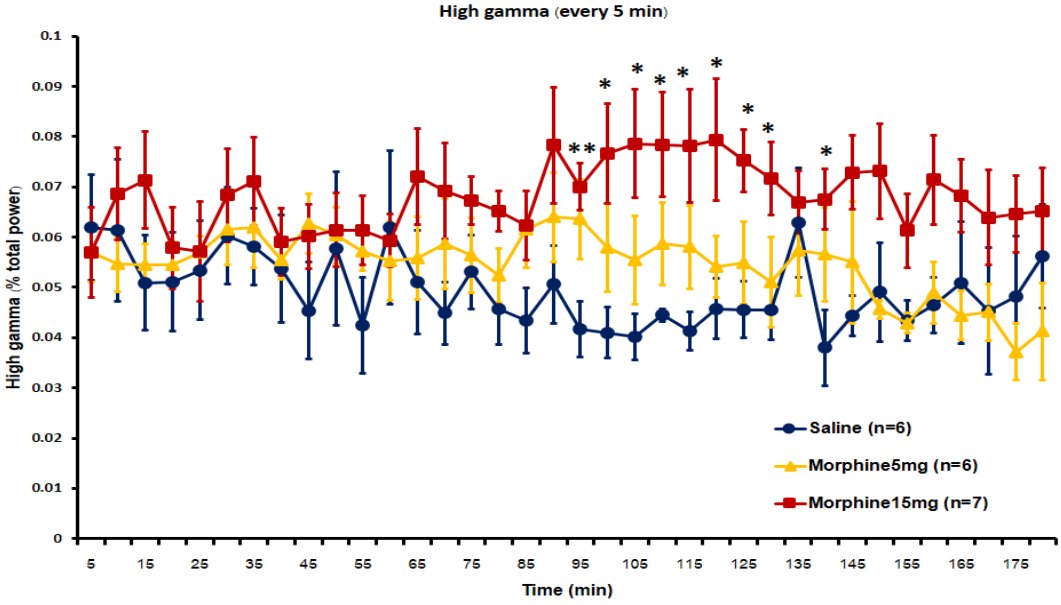


Figure 3.7 The average percent total power of high gamma range were analyzed every 5 min period after injection of morphine (5 and 15 mg/kg) or saline. Data were compared with that of saline control group using one-way ANOVA followed by Tukey's *post hoc* test. *, **: $p < 0.05$ and $p < 0.01$, respectively.

3.4.2.2 Correlation between striatal gamma power and locomotor activity following acute morphine administration

Regression analyses were performed to evaluate the correlation between striatum low and high gamma power and averaged speed (Fig. 3.8A) or averaged max speed (Fig. 3.8B) following morphine administration. The results showed no significant correlation between low gamma power and these two parameters of locomotor activity. However, the results showed significant correlations between high gamma power and these two parameters (averaged speed: 5 mg/kg morphine [$R^2 = 0.59$, $P < 0.001$], 15 mg/kg morphine [$R^2 = 0.34$, $P < 0.01$] and averaged max speed: 5 mg/kg morphine [$R^2 = 0.61$, $P < 0.001$], 15 mg/kg morphine [$R^2 = 0.21$, $P < 0.01$]).

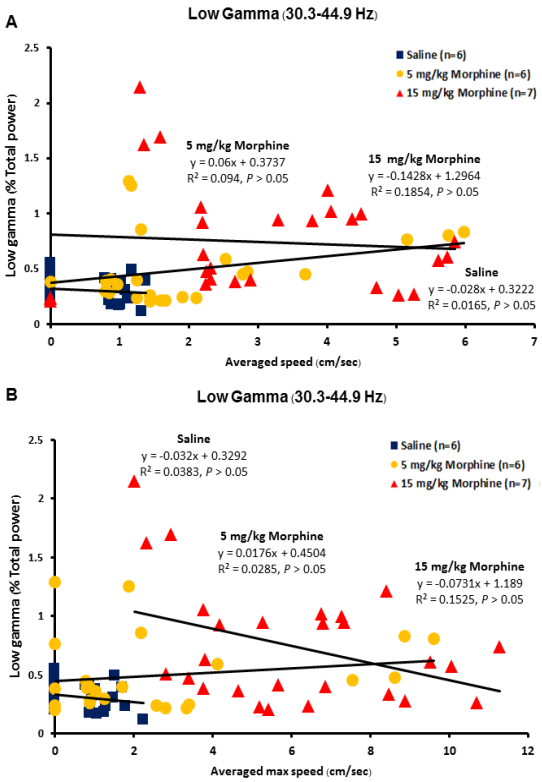


Figure 3.8 Correlations between striatum low gamma power and locomotor activity during acute morphine administration. Regression analyses between striatum low gamma oscillation and averaged speed and (A) averaged max speed (B).

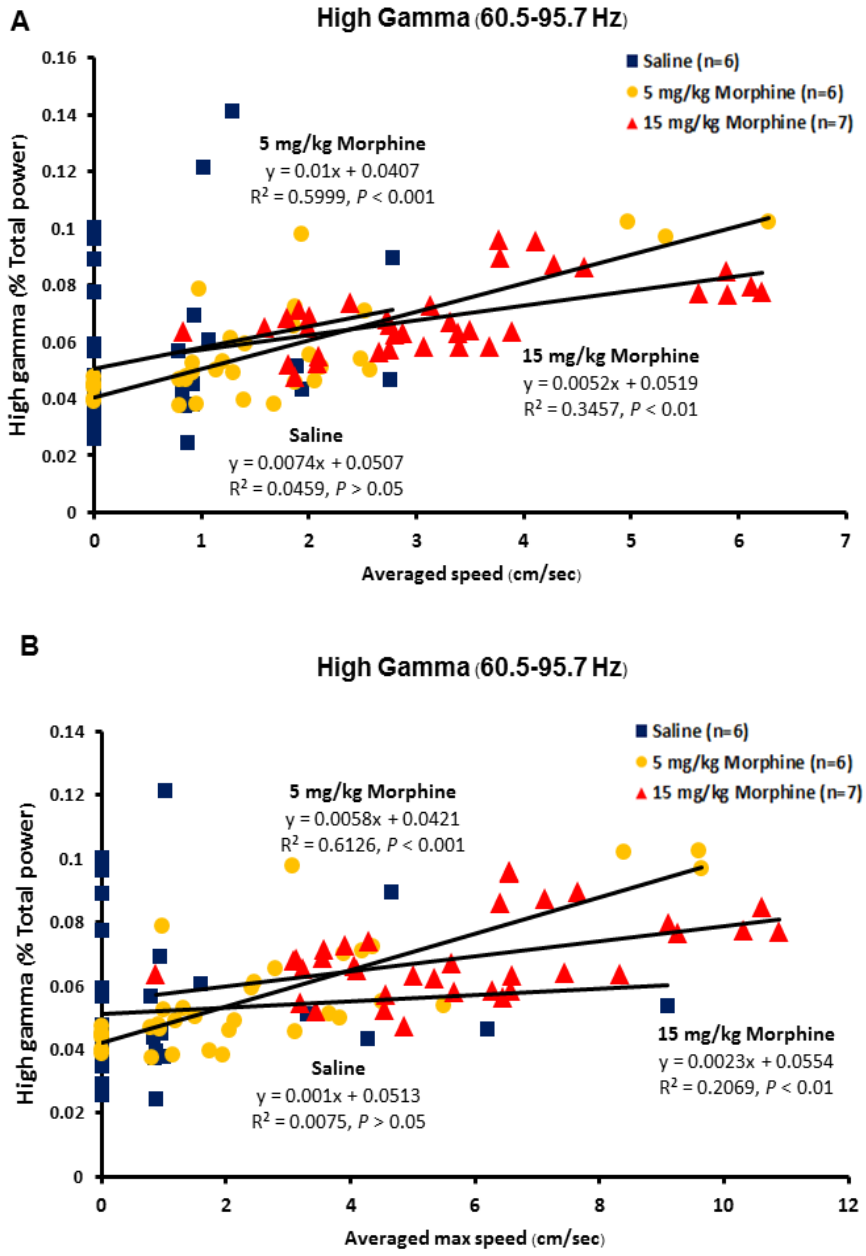


Figure 3.9 Correlations between striatum high gamma power and locomotor activity during acute morphine administration. Regression analyses between striatum high gamma oscillation and averaged speed (A) and averaged max speed (B).

3.4.3 Changes in LFP in the NAc following acute morphine administration

Following morphine administration, LFP signals in the NAc were recorded and representative raw LFP tracings were displayed. The overview of the signals caught the oscillatory character induced by morphine (Fig. 3.10B). Mainly, morphine injection appeared to increase fast brain wave. Relatively more fast waves induced by morphine were superimposed in raw LFP signals. Therefore, frequency analysis was performed for LFP power spectrum (% total power). Morphine appeared to increase low (30.3-44.9 Hz) and high (60.5-95.7 Hz) gamma power (Fig. 3.10A).

3.4.3.1 Time-course effects of acute morphine administration on low and high gamma power in the NAc

Frequency analysis raw LFP of the NAc during a period of 30-40 min was conducted for percent total power**.** Statistical analysis also confirmed that significant increases in low gamma power was seen in both the group of 5 mg/kg [$F(2,16)= 28.363$; $P= 0.023$] and 15 mg/kg morphine [$F(2,16)= 28.363$; $P < 0.001$] (Fig. 3.11A). Frequency analysis of raw LFP recorded in the NAc during a period of 100-110 min and statistical analysis confirmed that significant increase in high gamma power was seen in the group of 15 mg/kg morphine [$F(2,16)= 7.160$; $P= 0.005$] (Fig. 3.11B). No significant difference was produced by 5 mg/kg morphine.

Gamma activities (low and high frequency ranges) were particularly monitored every 5 min following morphine administration. Gradual increase in low gamma power was observed (Fig. 3.12). Significant increase was clearly produced by high dose of morphine (15 mg/kg) from the 15^{th} min and peaked at 35^{th} min. Low gamma activity was maintained proximately at peaked level for almost 40 min. Therefore, low gamma power was gradually declined to control level at 150^{th} min (15 min F [2, 16] = 10.018, P = 0.001], 20 min F [2, 16] = 11.186, P = 0.001), 25 min F [2, 16] = 12.445, P = 0.001], 30 min F [2, 16] = 23.382, P < 0.001], 35 min F [2, 16] = 19.473, P < 0.001], 40 min F [2, 16] = 42.448, P < 0.001], 45 min F [2, 16] = 46.003, P < 0.001], 50 min F [2, 16] = 35.605, P < 0.001], 55 min F [2, 16] = 36.171, P < 0.001], 60 min F [2, 16] = 14.556, P < 0.001], 65 min F [2, 16] = 24.201, P < 0.001], 70 min F [2, 16] = 11.025, P = 0.001], 75 min F [2, 18] = 10.686, P = 0.001],

80 min F [2, 16] = 18.805, P < 0.001], 85 min F [2, 16] = 8.662, P = 0.003], 90 min F [2, 16] = 8.351, P = 0.002], 95 min F [2, 16] = 8.349, P = 0.003], 100 min F [2, 16] = 10.660, P = 0.002], 105 min F [2, 16] = 7.682, P = 0.004], 110 min F [2, 16] = 5.597, P = 0.012], 120 min F [2, 16] = 8.647, P = 0.011], 125 min F [2, 16] = 18.420, P < 0.001], 140 min F [2, 16] = 8.806, P = 0.002], 145 min F [2, 16] = 5.433, P = 0.017] and 150 min F [2, 16] = 3.903, P = 0.043]). Significant increase in low gamma power induced by low dose of morphine (5 mg/kg) was also seen in lower magnitude and narrower time period (25 min F [2, 16] = 12.445, P = 0.002], 30 min F [2, 16] = 23.382, P = 0.042], 40 min F [2, 16] = 42.448, P = 0.003] and 50 min F [2, 16] = 35.605, P = 0.035]). In contrast, the increased high gamma activity was found to be a slower response to 15 mg/kg morphine administration (Fig. 3.13). From control level, significant increase in high gamma power began at the 80th min. Therefore, progressive increase in high gamma power was seen until the 175th min (80 min F [2, 16] = 6.033, P = 0.037], 95 min F [2, 16] = 3.916, P = 0.034], 100 min F [2, 16] = 3.557, P = 0.042], 105 min F [2, 18] = 4.994, P = 0.016], 110 min F [2, 16] = 5.491, P = 0.012], 115 min F [2, 16] = 4.652, P = 0.020], 125 min F [2, 16] = 6.251, P = 0.008], 130 min F [2, 16] = 5.336, P = 0.020], 140 min F [2, 16] = 5.028, P = 0.015], 145 min F [2, 16] = 6.206, P = 0.011], 150 min F [2, 16] = 5.562, P = 0.012], 155 min F [2, 16] = 4.288, P = 0.026], 160 min F [2, 16] = 4.846, P = 0.018], 165 min F [2, 16] = 5.259, P = 0.019], 170 min F [2, 16] = 5.769, P = 0.013] and 175 min F [2, 16] = 3.793, P = 0.047). There was no significant difference induced by 5 mg/kg morphine.

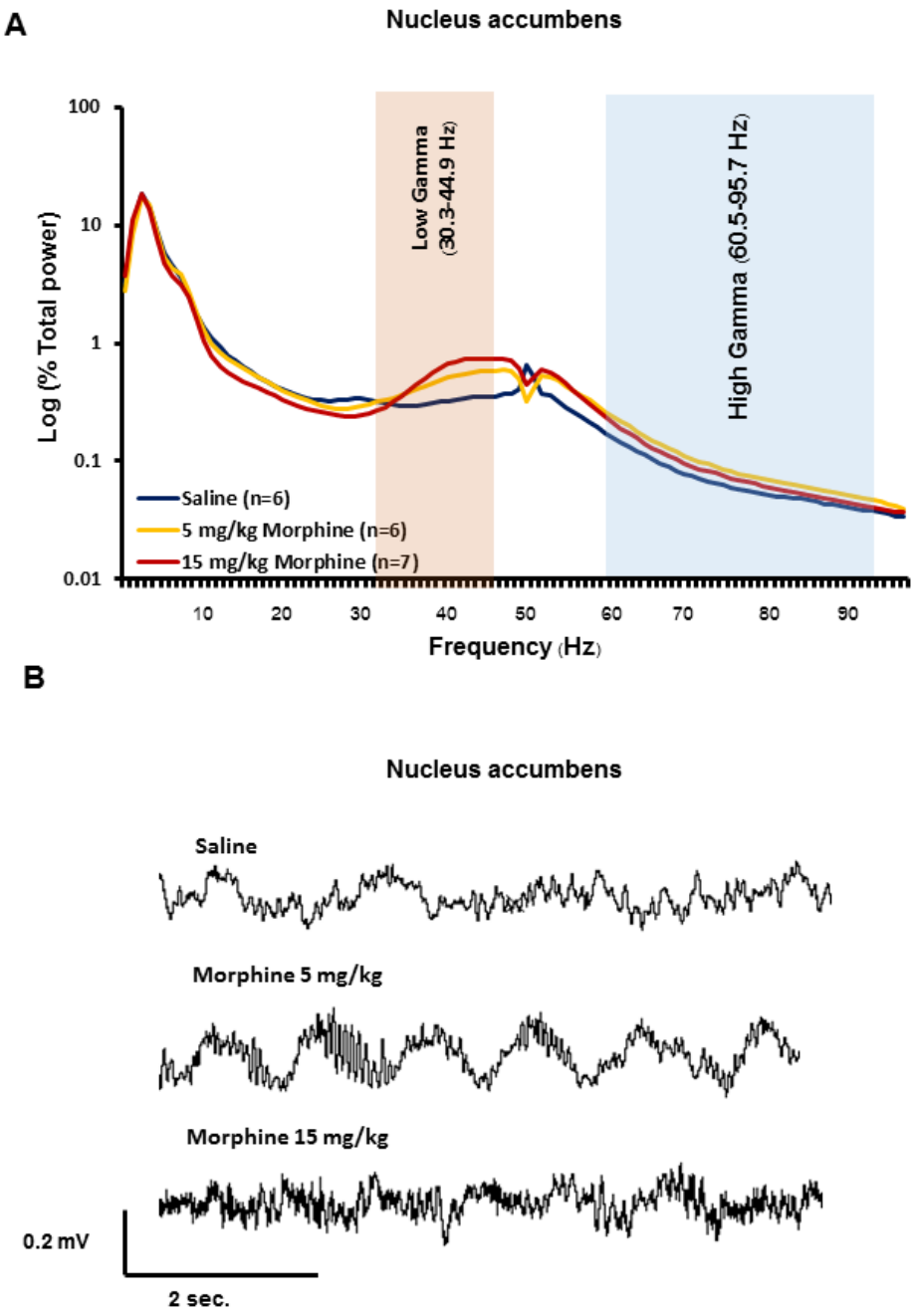


Figure 3.10 Frequency analyses following acute morphine administration. (A) Power spectrums of nucleus accumbens (NAc) local field potential (LFP) are expressed in frequency domain. (B) Raw striatum LFP signals recorded from representative mice that received saline, 5 mg/kg morphine and 15 mg/kg morphine were displayed in time-domain.

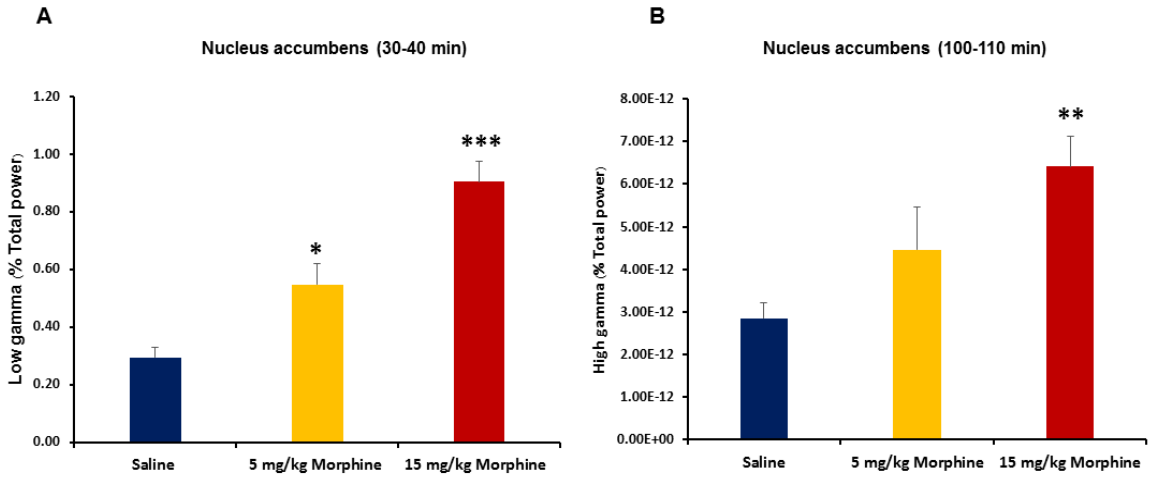


Figure 3.11 Frequency analyses following acute morphine administration. (A) Averaged percent total power of low gamma range of nucleus accumbens (NAc) 30-40 min. (B) Averaged percent total power of high gamma range of NAc 100-110 min are expressed as mean= ± S.E.M. * $p < 0.05$ compared with saline control group (one-way ANOVA followed by Tukey's *post hoc* test).

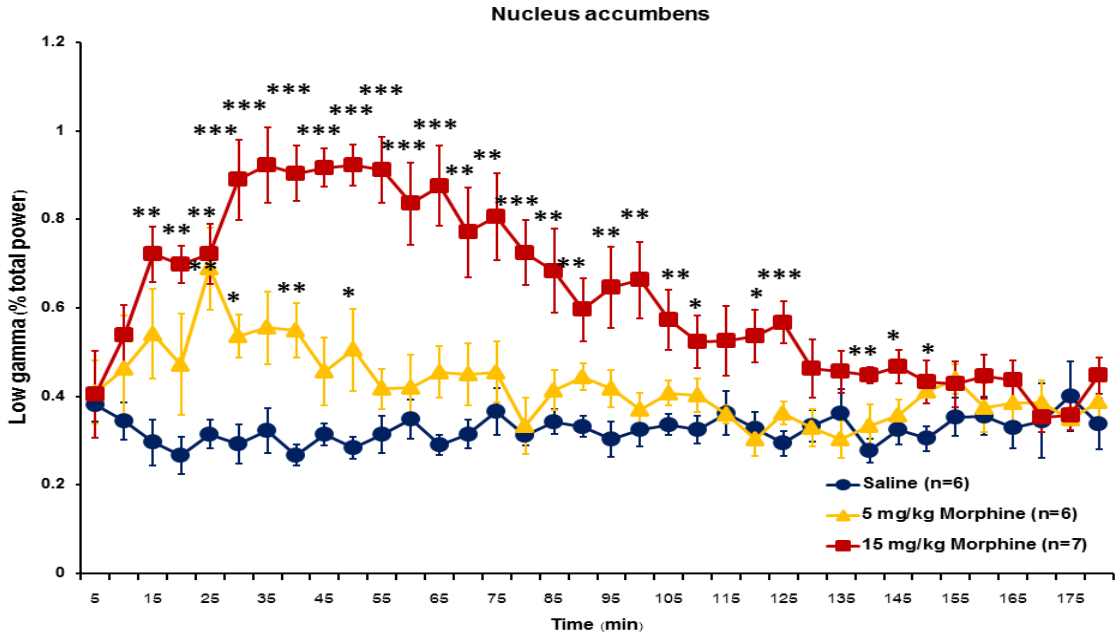


Figure 3.12 The average percent total powers of low gamma range in nucleus accumbens (NAc) were analyzed every 5 min period after the injection of morphine (5 and 15 mg/kg) or saline. Data were compared with that of saline control group using one-way ANOVA followed by Tukey's *post hoc* test. *, **, ***: $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively.

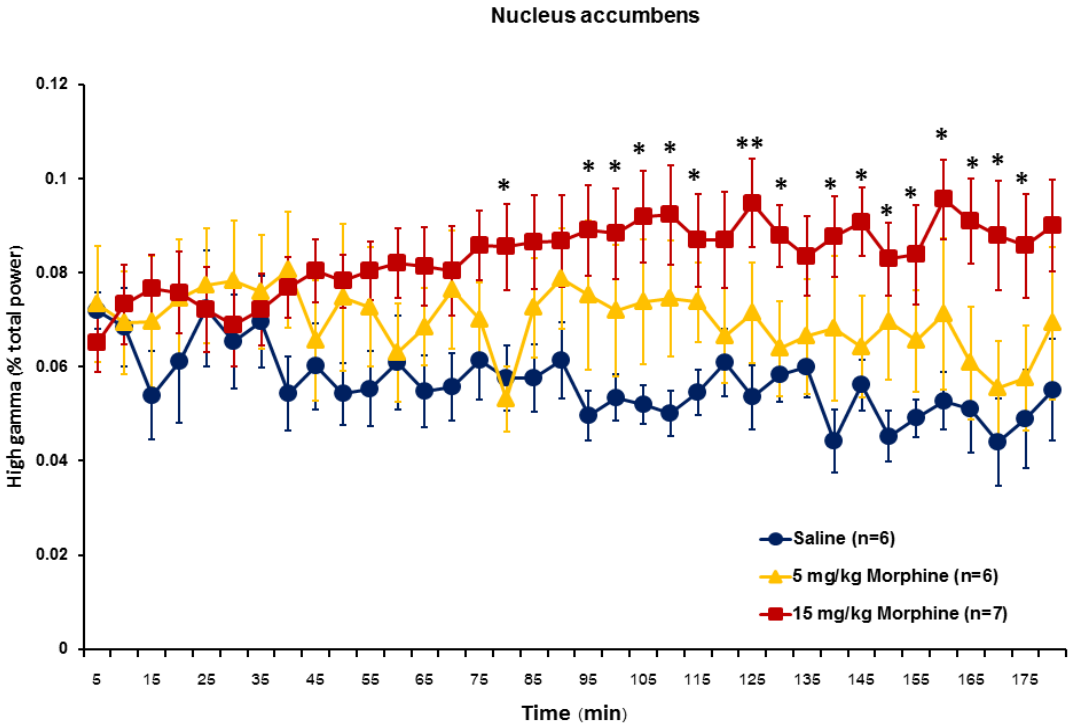


Figure 3.13 The average percent total power of high gamma range of nucleus accumbens (NAc) were analyzed every 5 min period after the injection of morphine (5 and 15 mg/kg) or saline. Data were compared with that of saline control group using one-way ANOVA followed by Tukey's *post hoc* test. *, **: $p < 0.05$ and $p < 0.01$ respectively.

3.4.3.2 Correlations between NAc gamma power and locomotor activity following acute morphine administration

Data were calculated from signals recorded in the NAc during 30-40 min and 100-110 min period for correlation between low or high gamma power, respectively, and locomotor activity. The results showed no significant correlation between gamma power (both low and high) and these two parameters of locomotor activity (Fig. 3.14A-D). The average speed and max average speed of locomotor activity did not predict gamma power in NAc.

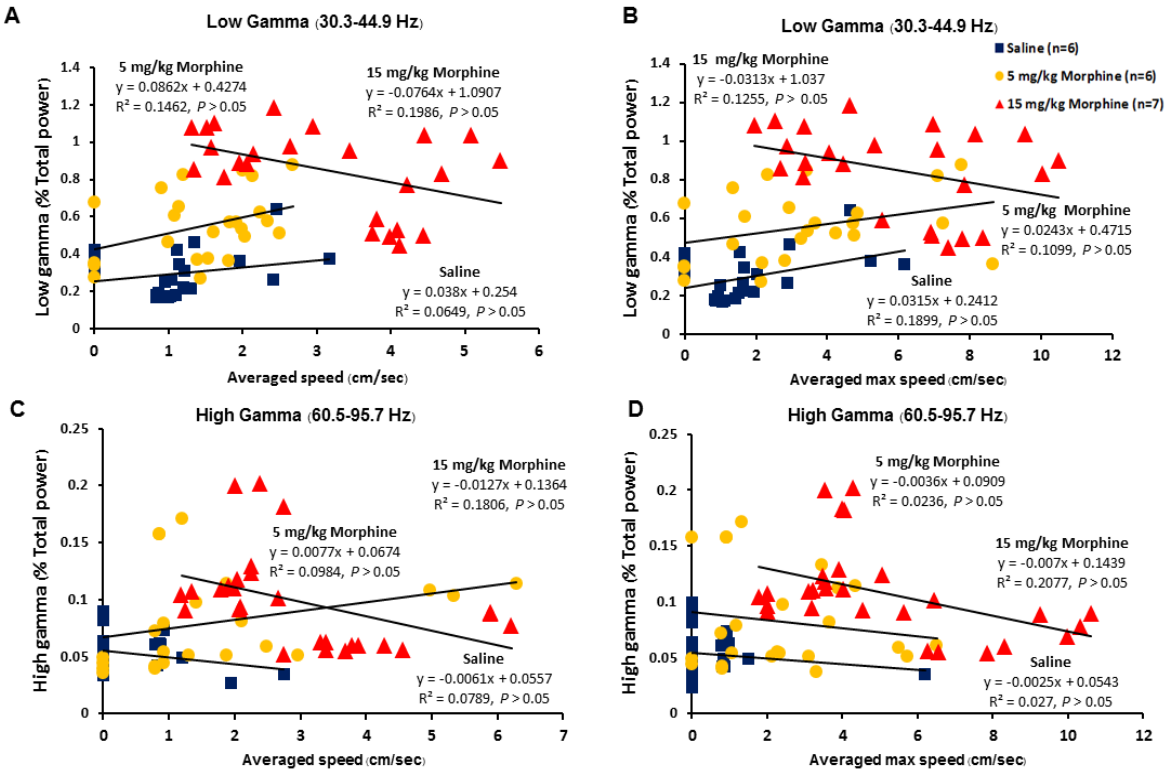


Figure 3.14 Correlations between nucleus accumbens (NAc) gamma power (both low and high) and locomotor activity following acute morphine administration. Regression analyses between low gamma oscillation and averaged speed (A) and averaged max speed (B). Regression analyses between high gamma oscillation and averaged speed (C) and averaged max speed (D).

3.5 Discussion and conclusion

3.5.1 Effects of morphine administration on locomotor activity

Drugs of abuse, such as opiates induce enhanced locomotor activity (Kalivas and Duffy, 1987). These results showed that morphine dose dependently increased averaged speed and averaged max speed in comparison to saline control group. Locomotor speed and averaged max speed were significantly increased in high dose of morphine. No significant change was induced by low dose of morphine for both locomotor parameters. These results are in agreement with those of previous studies (Becker et al., 2000; Yoo et al., 2003). Previous report suggested that the μ-opioid receptor plays an important role in modulating the acute locomotor activity induced by morphine (Yoo et al., 2003). However, some research indicated that dopamine also plays an important role in the locomotor activity. For example, dopamine-deficient mice were unable to produce a normal locomotor response but with high dose of morphine, dopamine- deficient mice were found to significantly increase locomotor activity (Hnasko et al., 2005). This study indicated that morphine can stimulate locomotor through a dopamine. Anyway, previous study even showed that no relationship between morphine-induced mesolimbic dopamine release and locomotion (Murphy et al., 2001). Afterward, reduced locomotor activity was seen as a result of either dopamine D1 (Tran et al., 2005) or D2 (Tran et al., 2002) receptor knockout (D1R-KO or D2R-KO respectively) in the nigrostriatal system. These previous findings suggested that locomotor activity induced by morphine was mediated by opiate receptor through an indirect dopaminergic mechanism (Zarrindast and Zarghi, 1992).

3.5.2 Oscillations in the striatum and the NAc in response to acute morphine administration

In the present study, morphine administration clearly affected brain oscillation in the striatum and NAc. The present data demonstrated some modifications of low and high gamma oscillation in the striatum and the NAc induced by morphine administration in mice. It has been well established that most addictive drugs produce their effects through activity of the dopamine neurotransmitter system as a common mechanism (Bozarth and Wise, 1981; Hnasko et al., 2005). Their effects on the dopamine system were dominant as the administration of these drugs was found to increase midbrain dopamine neuron firing (Gysling and Wang, 1983) and dopamine release preferentially in the NAc (Di Chiara and Imperato, 1988). In terms of mechanism of acute opiate action, the activation of dopamine cells induced by opiates could be mediated indirectly through μ-opiate receptors located on GABAergic midbrain interneurons that negatively regulate dopamine cell firing (Johnson and North, 1992). Activation of these inhibitory $G_{\alpha i}$-coupled μ-opiate receptors was found to withdraw the GABAergic tone from midbrain dopamine neurons which, in turn, resulted in increasing firing rate and the amount of dopamine released in the NAc (Hnasko et al., 2005). Moreover, additional research findings also demonstrated that morphine increased cell firing levels in both 2 dopaminergic origin areas, the VTA and the SNc (Gysling and Wang, 1983) and extracellular dopamine concentrations in 2 terminal dopaminergic areas, the NAc and the striatum (Lubetzki et al., 1982; Di Chiara and Imperato, 1988; Ghosh et al., 1998).

The striatum is among the main components of the basal ganglia complex. Its principal functions are primarily involved with motor function (Marsden, 1982). The nigrostriatal dopamine pathway (with dopamine cells locating in the SN projecting their axons to the striatum) is one of neural circuits that also has important roles in movement (Albin et al., 1989). Dopamine is produced by cells in the pars compacta of SN. Nigrostriatal axon terminals release dopamine into the striatum to produce an excitatory effect upon cells in the striatum (Albin et al., 1989). The dopamine deficits of this pathway are associated with movement disorders such as Parkinson's disease (Lotharius and Brundin, 2002). Basically, Parkinsonian

patients have considerable difficulties in initiation and termination of movement. Later, the involvement of this pathway in reward processes has been studied (Schultz, 1998). Therefore, the nigrostriatal dopamine pathway also plays a significant role in reward in addition to that of the mesolimbic and mesocortical dopamine pathways (Wise, 2009)

The present study clearly demonstrated that significant increases in low gamma power of the NAc were observed from the 25^{th} to 50^{th} min and 15^{th} to 150^{th} min following 5 mg/kg and 15 mg/kg morphine treatment, respectively. At the same time, significant increase in high gamma power of the NAc was observed from the 80^{th} to 175^{th} min following 15 mg/kg morphine treatment. The fact that gamma power occurred after morphine administration suggests that gamma oscillations did not simply reflect only the general state of the animals. The gamma range are found in many brain regions and it has been associated with a wide range of neural processes including working memory (Tallon-Baudry et al., 1999), perceptual (Singer and Gray, 1995) and motor function (Komek et al., 2012). However, regression analyses confirmed that the induction of low and high gamma oscillation in the NAc was not correlated with locomotor activity. Most of classical studies of reward function have focused on the activity of the ventral striatum, also known as the NAc (Spanagel and Weiss, 1999). In this study, the analysis of LFP in the ventral striatum revealed reward-associated gamma oscillations. Moreover, in the focus particular function of low and high gamma band, the low gamma was more related with reward than in the high gamma range (Kalenscher et al., 2010). In the ventral striatum, an increase in high gamma power was found during the approach toward a salient site in a T-maze while low gamma increase was detected abruptly at the reward site (Van der Meer and Redish, 2009). This report was consistent with that of Kalensher et al (2010). Additionally, an increase in low gamma on rewarded trials and a transient increase in high gamma before reward delivery were also demonstrated (Kalenscher et al., 2010). In contrast, low gamma was completely abolished upon reward receipt while high gamma only showed a transient increase to reward delivery (Berke, 2009). Presently, there are still discrepancies and the modulation of ventral striatum gamma by reward is still a topic of active research and is not explained by one normative theory. There

were shifts of low and high gamma activities both before and after reward delivery which might represent some shifts in the neural processing of rewards in the ventral striatum.

Anyway, the present study clearly demonstrated that the striatum significantly increased low and high gamma powers observed from the 15th to 65th min and 95th to 140th min, respectively, following 15 mg/kg morphine treatment. It has been well established for the rewarding properties of morphine (Hnasko et al., 2005; Sun et al., 2006). Furthermore, the striatum receives dopaminergic projections from the SN and is known as the brain regions primarily involved with motor function (Marsden, 1982). However, the stimulation of the SN, the brain areas that projects neural pathway mainly to the dorsal striatum, also produced rewarding effect (Wise, 1981). Previously, lesions of the dorsal striatum were found to reduce reward response to either cocaine or morphine (Suto et al., 2011). Taken together, these findings suggest some degrees of involvement of the dorsal striatum in drug reward and addiction. Therefore, it is likely that the enhanced gamma oscillation seen in the present study might be associated with reward induced by morphine treatment. Until recently, no direct link between gamma oscillation and reward has been established.

Following the administration of morphine, either reward or motor functions could be affected. Thus, regression analyses confirmed that the induction of low gamma oscillation was not correlated with locomotor activity. Only high gamma oscillation was correlated with locomotor activity. Previously, high gamma band activity in the striatum of human appeared during finger movement of the motor cortex (Huo et al., 2010) and in rats found that during voluntary behaviors (DeCoteau et al., 2007). In addition, in Parkinson disease models with the loss of dopamine-producing nerve cells also exhibited abnormally weakened high gamma frequency (Lemaire et al., 2012). Thus, modulation of high gamma oscillatory activity in the striatum may be a key feature of neural processing related to the control of voluntary behavior.

In conclusion, morphine administration was demonstrated to change LFP in the striatum and the NAc and locomotor activity in mice. The increase in high gamma activity was significantly correlated with locomotor activity in the striatum

but not in the NAc. These findings suggested that high gamma activity in the striatum might be specifically associated with motor function. Neither low nor high gamma oscillation in the NAc was correlated with locomotor activity. It is possible that these 2 ranges of gamma wave is associated with reward function. Moreover, the increased high gamma activity in the striatum was correlated with motor function. Altogether, these findings emphasized the involvement of the striatum and NAc in reward function.

CHAPTER 4

PATTERNS OF LOCAL FILED POTENTIAL OSCILLATION IN RESPONSE TO REPEATED MORPHINE ADMINISTRATION AND DURING MORPHINE WITHDRAWAL PERIOD IN CPP PARADIGM

4.1 Introduction

Drug addiction has produced negative impacts to health of world population for centuries, nevertheless the mechanisms by which particular drugs lead to addiction. Mainly, drug addiction is a chronically relapsing disorder characterized by compulsion to seek and take the drug and emergence of negative emotion state (e.g., anxiety, irritability) at some points of drug taking and abruption (Koob and Simon, 2009). In addition, intense drug craving can be reclaimed by only the presence of places or objects previously associated with drug use (De Vries and Shippenberg, 2002).

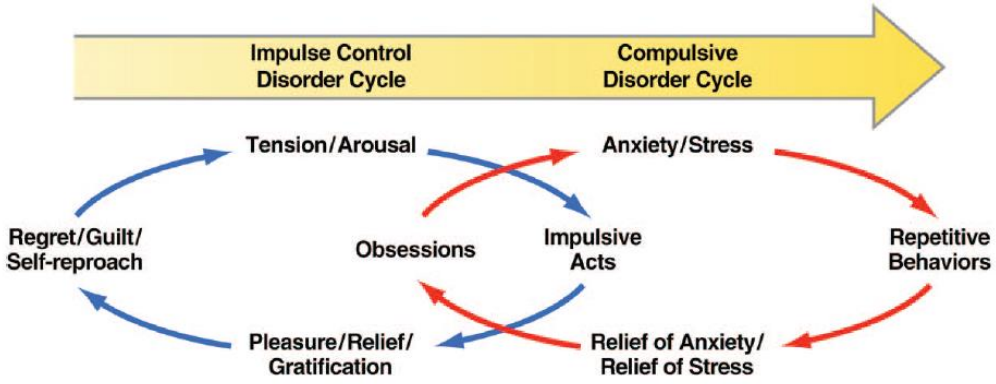


Figure 4.1 The diagram showing the stages of impulse control disorder and compulsive disorder cycles related to the sources of reinforcement (Koob, 2011).

Conceptually, addiction has been recognized with both impulsivity and compulsivity and neurobiological mechanisms necessary to drive individuals to do particular behavior (Koob, 2009). In impulse control, tension and arousal normally precede the impulsive act. Therefore, feeling pleasure, gratification or relief is likely to emerge during the act. After the act, there may or may not be regret or guilt. In compulsive behavior, thoughts are recurrent and persistent (obsessions) that induce extraordinary anxiety and stress and ultimately produce repetitive behaviors (compulsions) that are aimed at preventing or reducing distress (Figure 4.1) (Koob and Volkow, 2010; Koob, 2011)

However, knowledge of the neural mechanisms that underlie the transition from casual drug use to addiction is still inconclusive. Drug research in animal models is widely performed to determine the effects of drugs such as opiates, psychostimulants or alcohol. Although, no ideal animal model of addiction that would completely emulates the human condition, animal models do permit investigation of specific stages of the process of drug addiction (Koob, 2011)

Among the most important questions that have been asked, many current research have been conducted to focus on the involvement of the mesolimbic dopamine projection from the VTA to the NAc in incentive of drug-related stimuli, compulsive patterns of drug seeking, and the vulnerability to relapse that persists long after the disruption of drug use (Koob et al., 1998; Shippenberg and Elmer, 1998). Moreover, additional studies have been in attempt to examine a role of the striatum involved in addiction cycle (Ghosh et al., 1998). However, the activity of discrete brain regions induced by drug-related environment and condition remained largely to be investigated. The aim of this study was to observe LFP changes induced by morphine administration and the morphine-related environment (withdrawal).

4.2 Behavioral test

Conditioned place preference

The conditioned place preference (CPP) paradigm has been extensively used to study the rewarding and aversive effects of drugs. CPP paradigm is extensively used in conjunction with standard neuroscience techniques to elucidate the subjective effects of drugs (Tzschentke, 2007). In addition, CPP model can also be used as an effective tool to investigate the mechanisms underlying drug-induced reinstatement of drug seeking after extinction (Parker and Mcdonald, 2000). It is also popular in the study of addictive and potentially addictive drugs (Yan-Fang et al., 2007; Solinas et al., 2008).

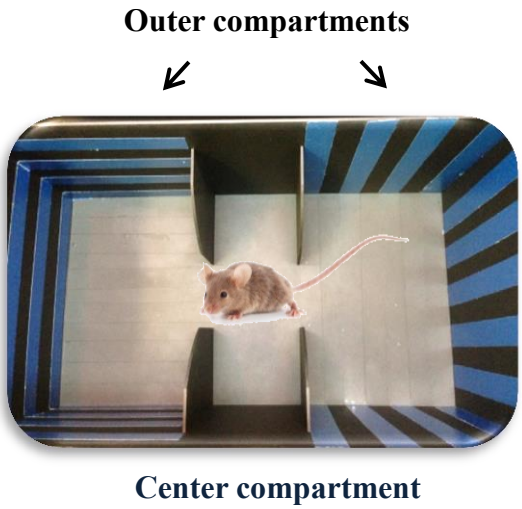


Figure 4.2 The characteristics of conditioned place preference (CPP) task.

Great number of different designs and apparatuses are used in this model. The basic characteristics of this task involve the association of a particular environment with drug treatment, followed by the association of a different environment with the absence of the drug (i.e., the drug's vehicle). A general form of this design consists of a three-compartment chamber which includes the outer compartments being designed to have different characteristics among them (e.g., white vs. black walls, horizontal grid vs. cross-grid flooring). The center compartment has no special characteristics and is not paired with a drug, and the gates

between the compartments can be opened to allow an animal to pass freely between them. In the present study, characteristics of CPP were designed for basic features. One outer compartment was characterized with black vertical lines of walls and the floor. Meanwhile, the other outer compartment was designed with black horizontal lines of walls and the floor.

The underlying learning process of CPP for incentive-driven behavior is assumed to be based on Pavlovian conditioning (Bardo and Bevins, 2000). Explanation invokes the seeking out of a place or stimulus complex which had been paired with the reinforcer. In this paradigm, the reinforcer, an unconditioned stimulus (UCS), exhibits some effects on the organism which elicits an unconditioned response (UCR). This is paired with the stimulus properties of the place, which become conditioned stimuli (CS). Gradually, the CS is automatically assumed 'incentive value' of their own, which leads the organism to 'seek' these out, or to 'prefer' them (Bindra, 1974; Bardo and Bevins, 2000; Spiteri et al., 2000; Huston et al., 2013).

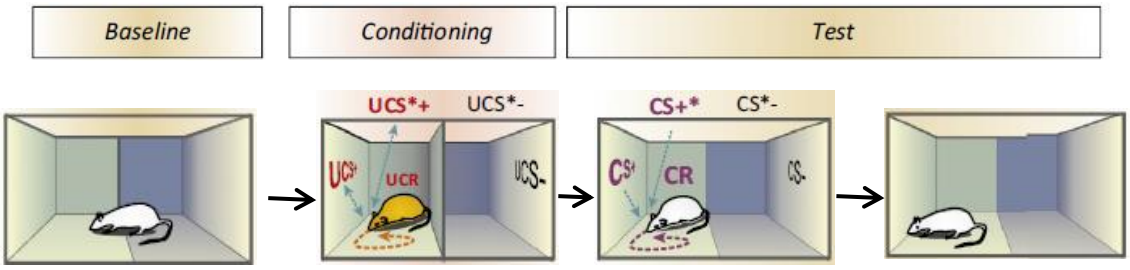


Figure 4.3 Drugs and natural reinforcers may induce an acute UCR. This can be conditioned to a UCS+/UCS*+. They become a CS+/CS*+ and may elicit CR similar to the UCR during the test (Huston et al., 2013). UCR; unconditioned behavioral response, UCS; unconditioned stimulus intra-maze, UCS*; unconditioned stimulus extra-maze, CS; conditioned stimulus intra-maze, CS*; conditioned stimulus extra-maze and CR; conditioned response.

4.3 The experimental procedure for recording of spontaneous LFP signal and CPP behavior during morphine administration and withdrawal

For experimental procedure and LFP recording, animals were subjected for repeated measures for 20 consecutive days using 3 phases of CPP paradigm (pre-conditioning phase, conditioning phase and post-conditioning phase). During pre-conditioning phase (day $1^{st} – 3^{rd}$), individual animals were placed into center apparatus for 20 min with free access to all compartments. Number of entry and time spent in each compartment were evaluated. Conditioning phase required a period of 10 days. Partitions were inserted to divide each compartments. For this phase, animals were divided into 2 groups as control and morphine groups. Effects of morphine administration were paired with specific condition confined to a fixed compartment (vertical line of walls and floor chamber or right side of apparatus) for 45 min. They received intraperitoneal injection of morphine (0.5, 1.0, 2.5, 5.0 and 15 mg/kg for day 1, 2, 3, 4 and 5-10, respectively). Saline-paired condition was confined to the other compartment (horizontal line of walls and floor chamber or left side of apparatus). Individual animals in saline group received saline injection paired with the opposite compartment to that of morphine. LFP signals were recorded during this phase. Post-conditioning phase required a period of 7 days when the partitions were removed from the CPP apparatus. Therefore, the two chambers were opened again. During this phase, each animal was placed into the center of the apparatus. They could explore and access the entire apparatus for 20 min without any restriction. Animal movement and LFP signals were simultaneously recorded. Number and time spent in each compartments were analyzed and frequency components of LFP were investigated.

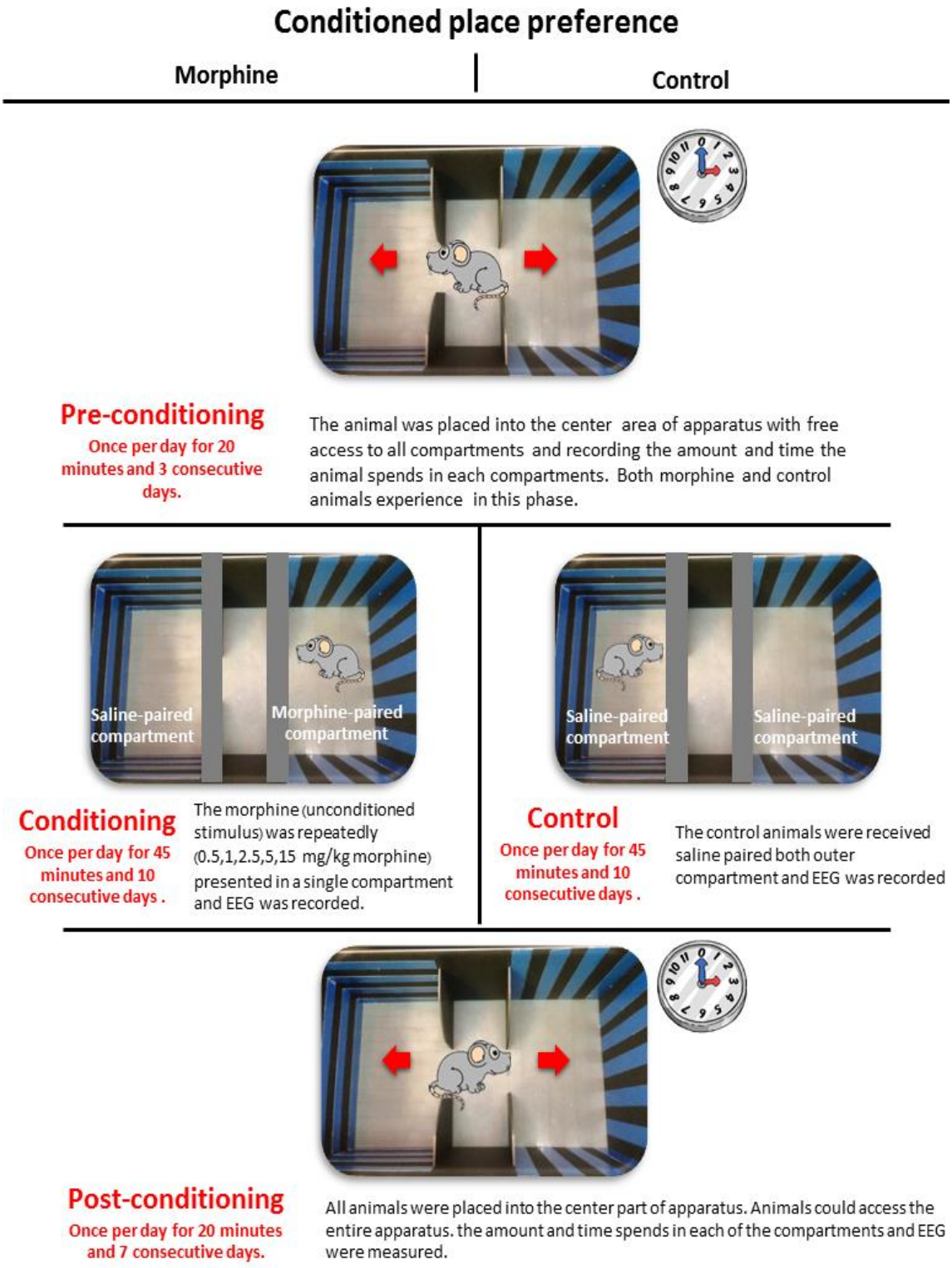


Figure 4.4 Experimental procedure for recording of spontaneous local field potential (LFP) signals and conditioned place preference (CPP) behavior following morphine administration and during exposure to morphine-related environment (withdrawal).

4.4 Statistical analysis

All data were averaged and expressed as mean ± S.E.M. Differences between the control and morphine groups were analyzed by using two-way analysis of variance (ANOVA) followed by multiple comparisons using Tukey's *post hoc* test. For CPP paradigm, data of post-condition were subtracted with that of the first day habituation. Therefore, differences between the control and morphine groups were analyzed by using *t-test*. Levels of significance were set at $p < 0.05$.

4.5 Results

4.5.1 Effects of morphine administration on locomotor activity

In parallel with LFP recording, spontaneous motor activity was also simultaneously recorded during pre-condition phase, condition phase and post-condition phase of CPP by using a webcam. The results showed that morphine increased averaged speed and averaged max speed in comparison to that of saline control group (Fig. 4.5A and B) during conditioning phase or repeated morphine. Therefore, two-way ANOVA revealed significant increases in averaged speed during condition5 [$F(1,87)= 6.434$; $P = 0.006$], condition10 [$F(1,87)= 6.434$; $P = 0.003$] and averaged max speed of condition10 [$F(1,87)= 1.381$; $P = 0.046$].

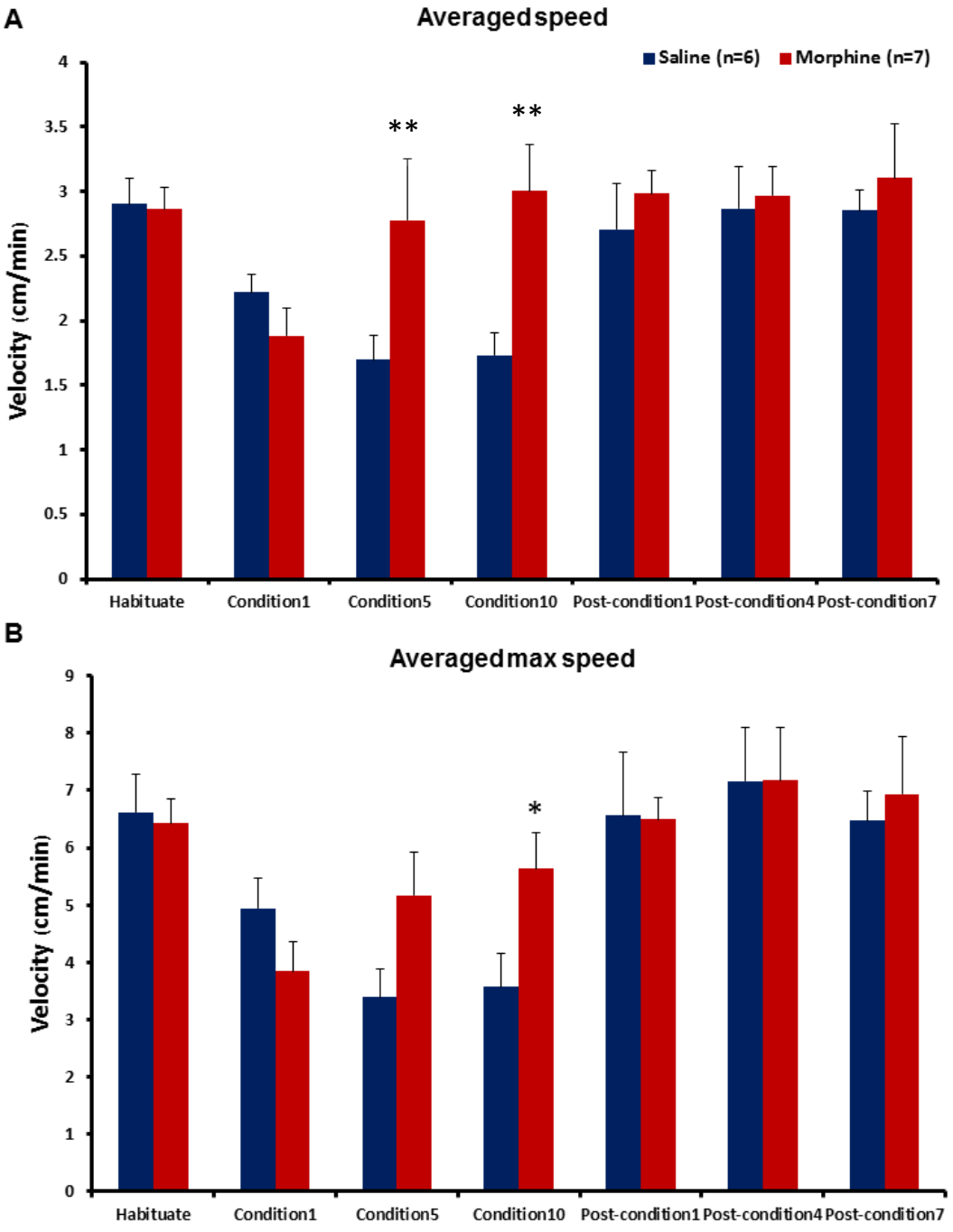


Figure 4.5 Spontaneous motor activity of animals during habituation, condition phase and post-condition phase of conditioned place preference (CPP). (A) Averaged speed and (B) Averaged max speed during 3 phase of CPP expressed as mean ± S.E.M. Data were analyzed using two-way ANOVA. *, ** : $p < 0.05$ and $p < 0.01$ compared with the saline control group respectively.

4.5.2 Effects of morphine administration on CPP behavior

During post-conditioning phase of CPP, number and time spent in each of the compartments were measured by using video records. CPP behavior was examined after restraining period of morphine condition. Following the repeated administration of morphine, the increases in number and time spent were seen for morphine-paired compartment (right side) in comparison to saline control group (Fig. 4.7A and B, respectively). Therefore, *t-test* revealed significant increases in number of entries during post-condition1 [$t_{(12)}$= -3.077; p = 0.012], post-condition4 [$t_{(12)}$= -2.426; p=0.036] and time spends of post-condition1 [$t_{(12)}$= -2.257; p = 0.048], post-condition4 [$t_{(12)}$= -3.240; p = 0.009] and post-condition7 [$t_{(12)}$= -2.943; p = 0.015] in morphine group.

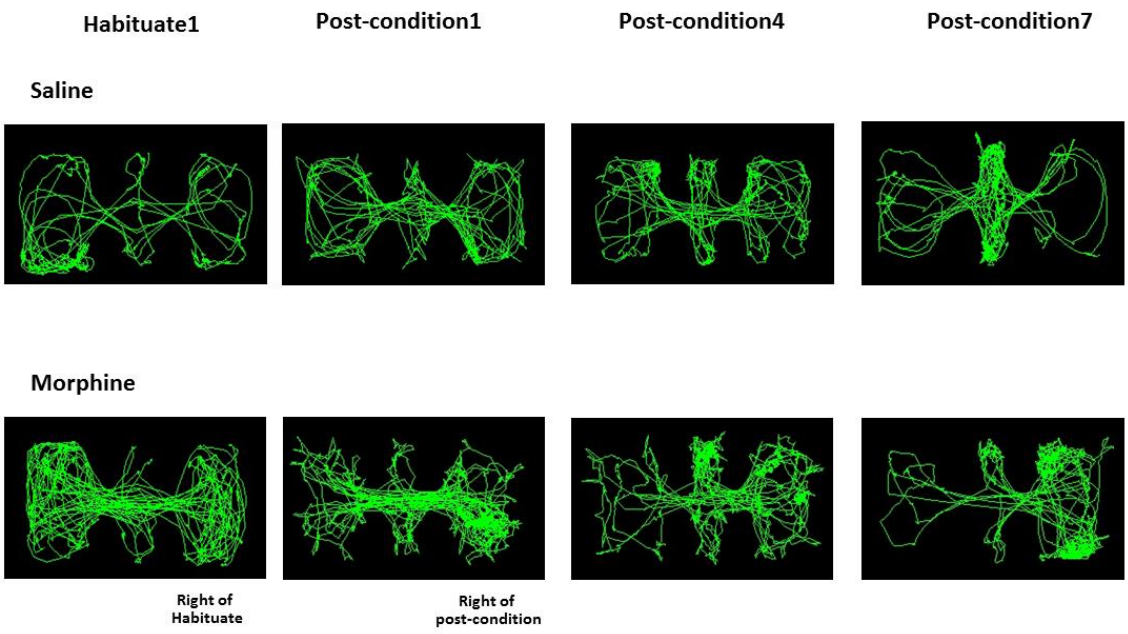


Figure 4.6 Tracking of spontaneous motor activity of animals during habituation and post-condition phase of conditioned place preference (CPP).

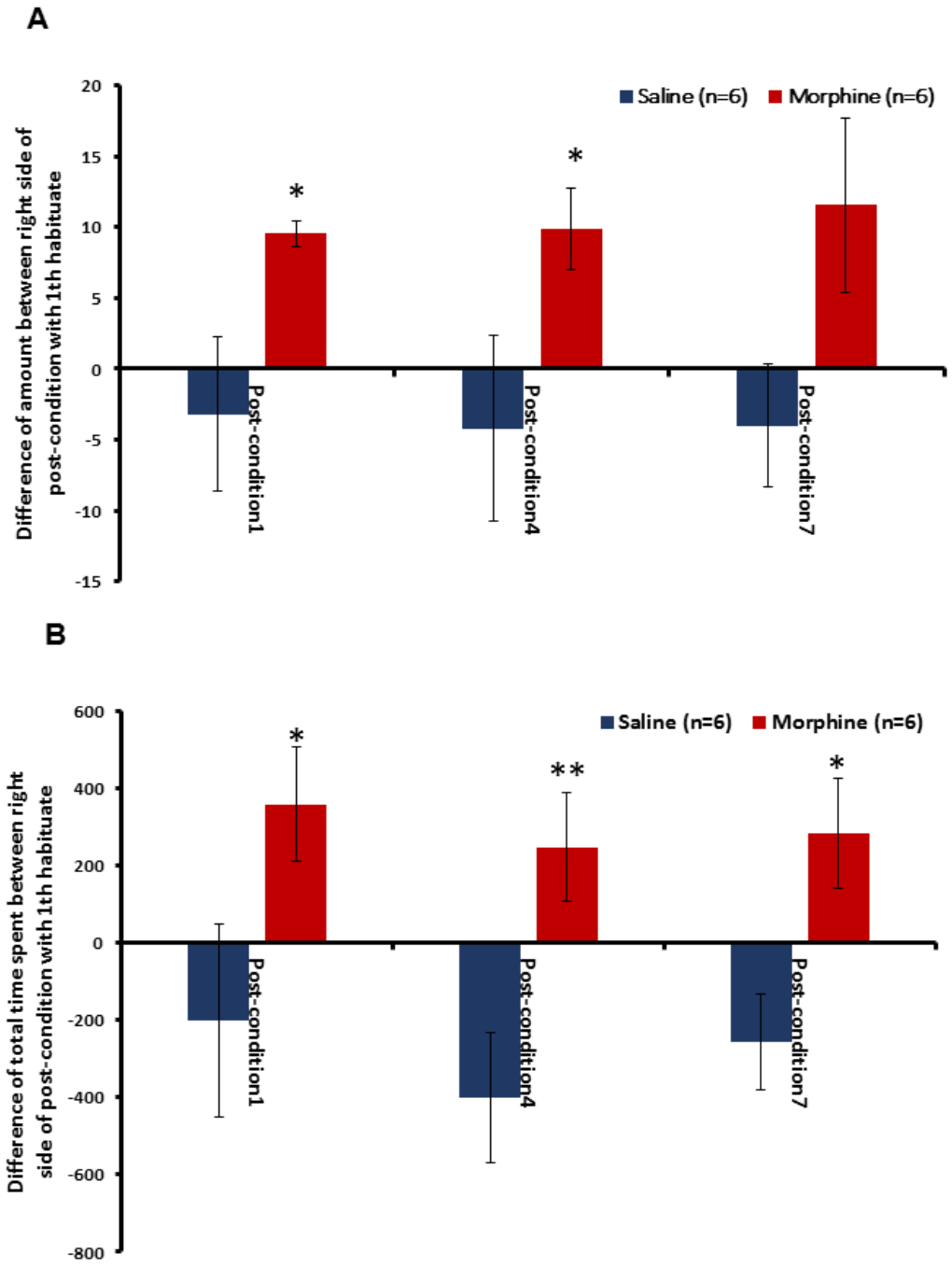


Figure 4.7 Behavioral measurement during post-conditioning phase of conditioned place preference (CPP). Number of entries (A) and time spent (B) of spontaneous motor activity in each compartment in CPP apparatus are shown. Differences between entry number and time spent in the right side during post-conditions with the 1^{th} habituation are expressed as mean ± S.E.M. *, ** : $p < 0.05$ and $p < 0.01$ compared with the saline control group respectively.

4.5.3 Changed in striatal LFP during repeated administration and withdrawal period of morphine

During conditioning phase, animals in morphine group received morphine at the dose 0.5, 1, 2.5, 5 mg/kg respectively on day 1-4, respectively, and the dose 15 mg/kg from day 5-10 while animals in control group received saline for 10 consecutive days. In each day, LFP signals from individual mice were continuously recorded for 45 minutes in each outer compartment. Comparisons between data of saline and morphine groups were made for each post-condition day. Prior to frequency analysis of LFP, signals were visually inspected for their general appearances. Spectrograms of the signals from the striatum during pre-condition, condition and post-condition were shown (Fig. 4.8). By using a gray scale code reference of power, it seemed the striatum had different activity of alpha oscillation between LFP spectrograms of pre-condition, condition and post-condition phase.

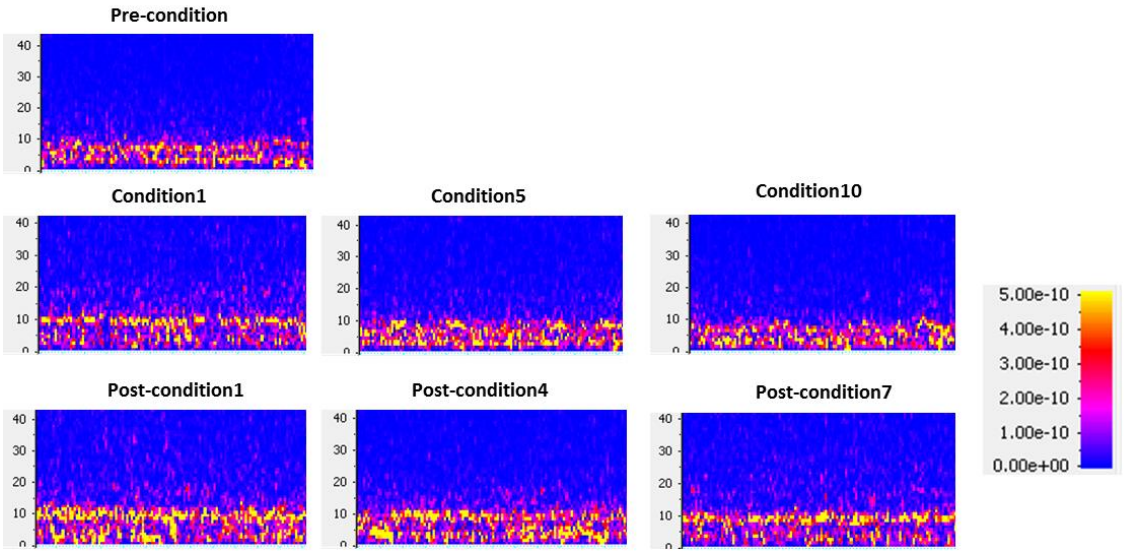


Figure 4.8 Representative spectrograms of local field potential (LFP) signals from the striatum. Signals were recorded from animals in 3 phases which included pre-condition, condition and post-condition.

Therefore, frequency analysis was performed to quantitate percent total power of the LFP in a range of alpha (9.7-12 Hz). Obviously, the lowest morphine treatment (morphine conditioning1; 0.5 mg/kg morphine) appeared to increase activity in a range of alpha oscillation. After that, alpha activity decreased during repeated morphine administration (morphine conditioning 5 and 10; 15 mg/kg morphine) (Fig. 4.9). Statistical analysis found that no significant increase in alpha power of morphine conditioning1 in comparison to control group. However, during repeated administration of morphine, significant decreases in alpha power were seen in morphine conditioning 5 [$F(1,87)= 0.594$; $P = 0.039$] and 10 [F(1,87)= 0.594; =0.014] in comparison to that of control group. In contrast, no significant change in percent total power of alpha wave was produced during post-conditioning or morphine withdrawal.

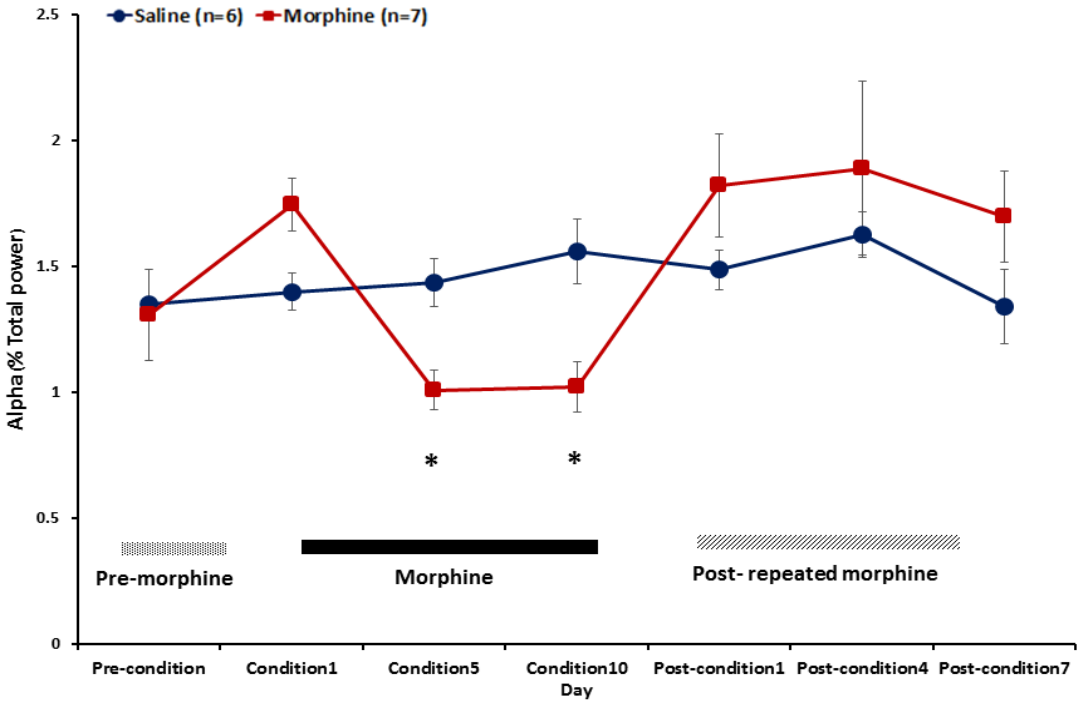


Figure 4.9 Frequency analysis of alpha power range in striatum during pre-conditioning, conditioning and post-conditioning phase of conditioned place preference (CPP) apparatus treated. Data was expressed as mean ± S.E.M. * : $p < 0.05$ compared with control group.

Moreover, regression analyses were performed to evaluate the correlation between striatum alpha power and averaged speed (Fig. 4.10 A and 4.10 C) or averaged max speed (Fig. 4.10 B and 4.10 D) during condition 5 and condition 10 of CPP apparatus. The results showed no significant correlation between alpha power and these two parameters of locomotor activity during condition 5 and condition 10 of CPP apparatus.

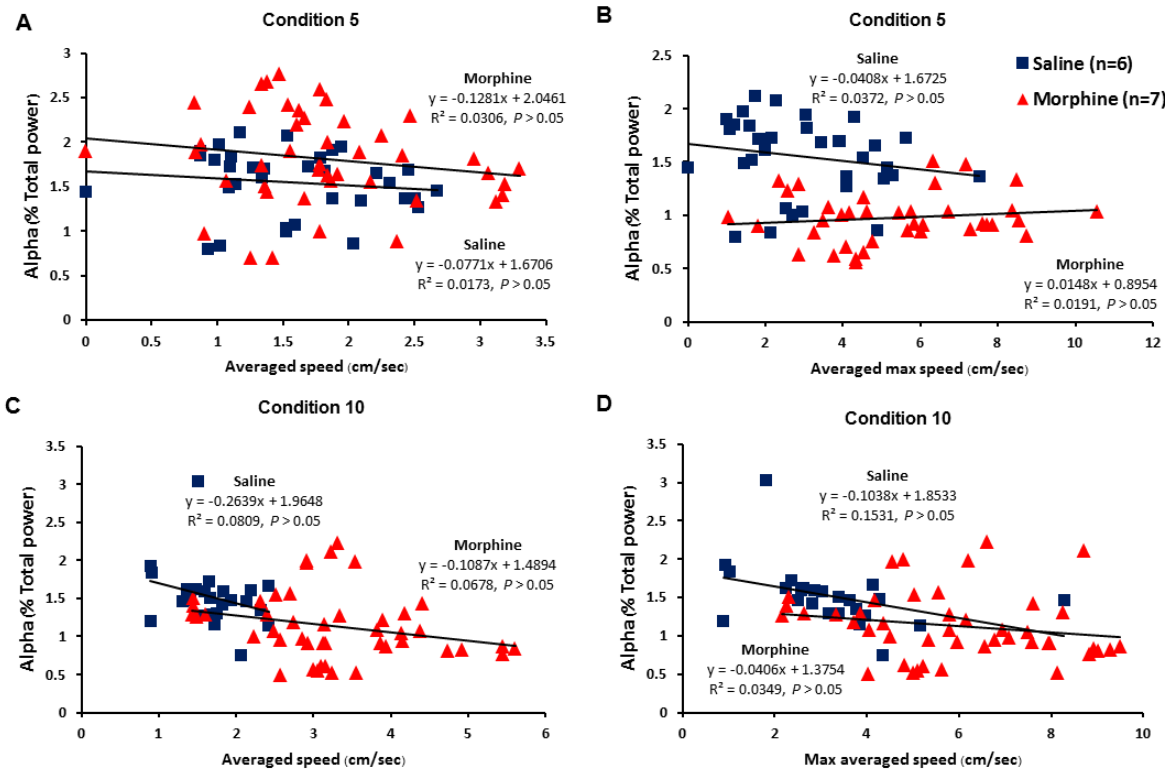


Figure 4.10 Correlations between striatum alpha power and locomotor activity during condition 5 and condition 10 of CPP apparatus. Regression analyses between alpha oscillation and averaged speed of condition 5 (A) and condition 10 (C) and averaged max speed of condition 5 (B) and condition 10 (D).

4.5.4 Changed in NAc LFP during repeated administration and withdrawal period of morphine

Spectrograms of the signals from NAc during pre-condition, condition and post-condition were shown (Fig. 4.11). It seemed NAc had also different activity of alpha oscillation between LFP spectrograms of pre-condition, condition and post-condition phase. Thereafter, frequency analysis was performed to quantitate percent total power of the LFP in a range of alpha (Fig. 4.12). The results showed that the lowest morphine treatment appeared to increase activity in a range of alpha oscillation. However, alpha activity decreased during repeated morphine administration. Additionally, animals in morphine group show that alpha power increased about two fold in comparison to control group. Statistical analysis confirmed that significant increase in alpha power of post-condition1 [$F(1,87) = 26.601$; $P < 0.001$], post-condition4 [$F(1,87) = 26.601$; $P < 0.001$] and post-condition7 [$F(1,87)= 26.601$; $P < 0.001$] comparison to control group and post-condition4 $F(6,87) = 8.726$; $P < 0.001$], post-condition7 [$F(6,87)= 8.726$; $P = 0.01$] comparison to morphine group. In contrast, no significant change in percent total power of alpha wave was produced during conditioning.

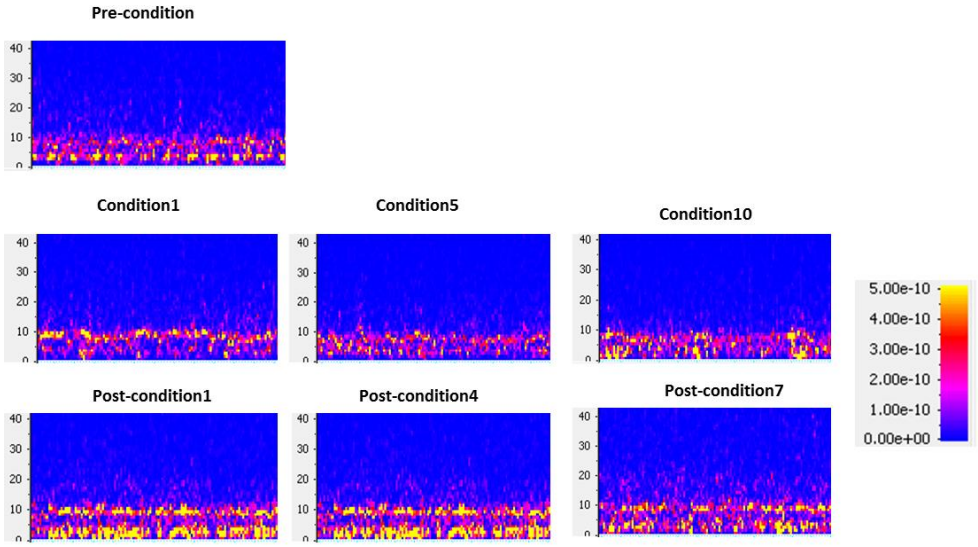


Figure 4.11 Representative spectrograms of local field potential (LFP) signals from nucleus accumbens (NAc). Signals were recorded from animals in 3 phases which included pre-condition, condition and post-condition.

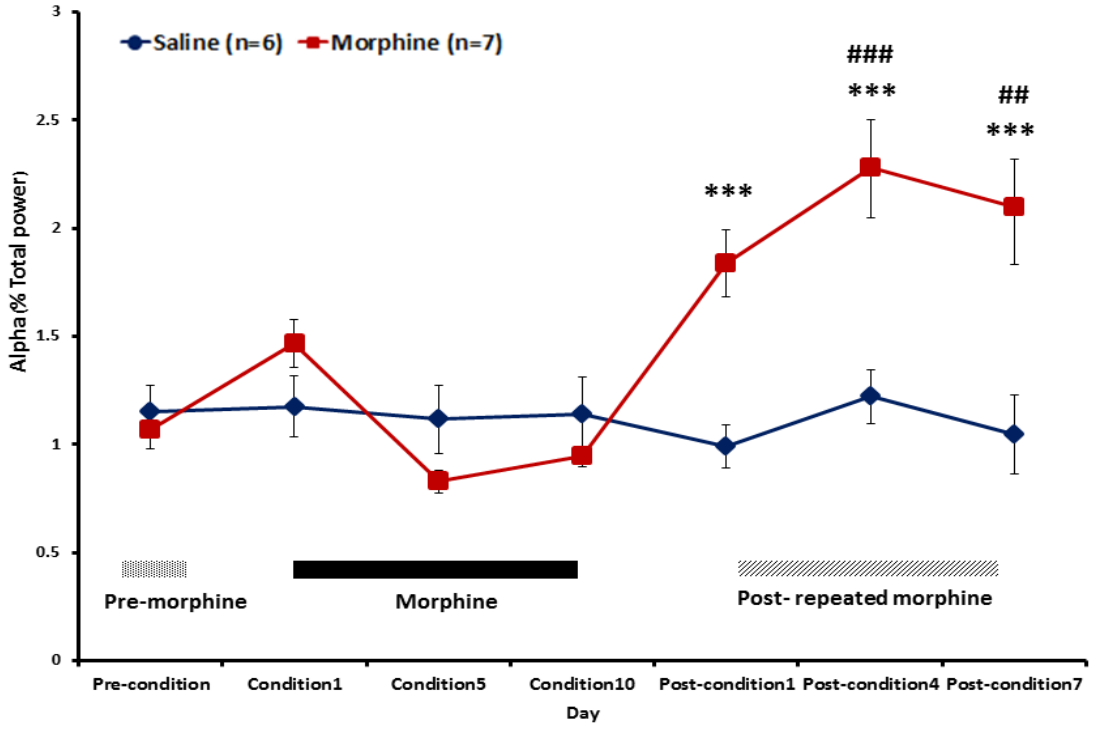


Figure 4.12 Frequency analysis of alpha power range in nucleus accumbens (NAc) during pre-conditioning, conditioning and post-conditioning phase of conditioned place preference (CPP) apparatus treated. Data was expressed as mean ± S.E.M. *** : $p < 0.001$ compared with control group. ##, ###: $p < 0.01$ and $p < 0.001$ respectively compared with pre-conditioning (pre-morphine).

Addition, regression analyses were performed to evaluate the correlation between NAc alpha power and averaged speed (Fig. 4.13 A, 4.13C and 4.13 E) or averaged max speed (Fig. 4.13B, 4.13D and 4.13 F) during post-condition 1, post-condition 4 and post-condition 7 of CPP apparatus. The results showed no significant correlation between alpha power and these two parameters of locomotor activity during post-condition 1, post-condition 4 and post-condition 7 of CPP apparatus.

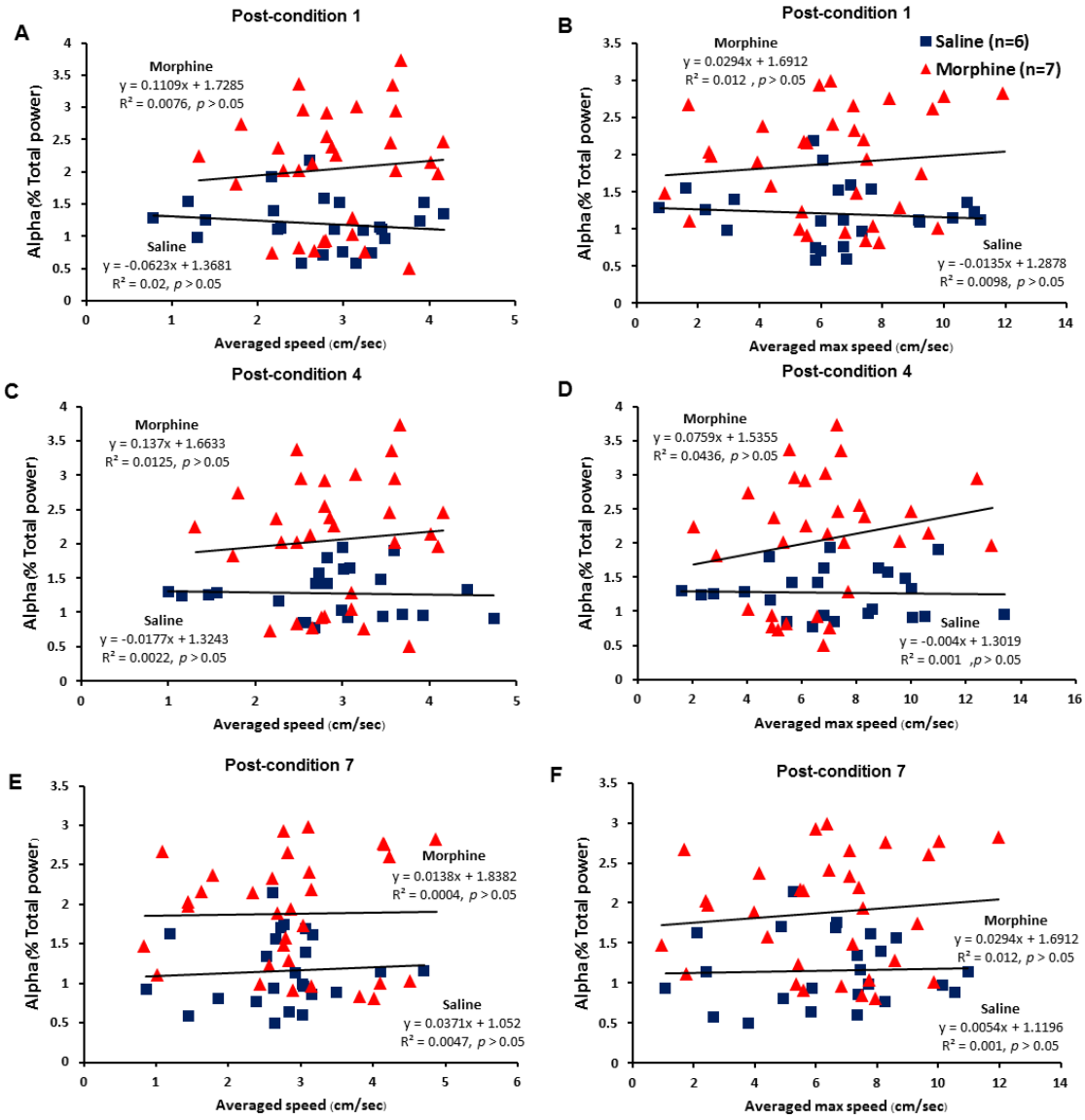


Figure 4.13 Correlations between nucleus accumbens (NAc) alpha power and locomotor activity during post-condition 1, post-condition 4 and post-condition 7 of conditioned place preference (CPP). Regression analyses between alpha oscillation and averaged speed of post-condition 1 (A), post-condition 4 (C), post-codition 7 (E) and averaged max speed of of post-condition 1 (B), post-condition 4 (D), post-codition 7 (F).

4.6 Discussion and conclusion

4.6.1 Effects of morphine administration on CPP behavior

Conditioned place preference, a behavioral task often used as a screening tool for drug abuse potential, has been also used to measure memory or learning of simple stimulus-reward associations (McIntyre et al., 1998). Therefore, this study used CPP paradigm to investigate chronic morphine administration in mice. It was found that following chronic morphine administration significantly increased both amount of entry and time spent in morphine-pair compartment side. These findings support numerous studies in that morphine induces a conditioned preference for the place in which it has been administered in rats (Mucha and Iversen, 1954; De Fonseca et al., 1995; Olmstead and Franklin, 1996; Shippenberg et al., 1996; Popik and Danysz, 1997) and in mice (Bespalov et al., 1999; Belzung and Barreau, 2000; Gong et al., 2010).

It is generally agreed that morphine-induced CPP depends critically on the dopamine mesolimbic system (Manzanedo et al., 2001). Dopamine antagonist (Acquas et al., 1989; Suzuki et al., 1993) or 6-hydroxydopamine (6-OHDA) lesion of VTA or NAc abolished this effect (Spyraki et al., 1983; Wang et al., 2003). Additionally, injection of morphine into the VTA produced CPP (Phillips and LePiane, 1890; Olmstead and Franklin, 1997) and there are some reports showing that unilateral microinjection of the μ-opioid agonist DAMGO into the VTA produced CPP (Bals-Kubik et al., 1993). These suggest that μ-opiate receptors, located in the VTA, play an important role in mediating the reinforcing effects of morphine. Moreover, NAc is also the brain area that mediates the rewarding effects of morphine. Previously, it was reported that unilateral injections of morphine into the NAc produced CPP in rats (Van Der Kooy et al., 1982). Thus, it has been suggested that activating of opioid receptors within the NAc can be positively reinforcing (us reviewed by Zarrindast and Rezayof, 2007). On the other hand, direct injection of morphine or opioid agonists into other areas, such as the caudate-putamen or the amygdala failed to produce CPP (Van Der Kooy et al., 1982). However, some CPP studies suggested that the central amygdala (CeA) may also play an important role in

reward function produced by drugs of abuse (O'Dell et al., 1999). Considering the anatomy and functions of CeA, as the CeA receives dense dopamine efferents from the VTA (Kilts and Anderson, 1987). It is likely to have an important role in the control of motivation and the effects of drug conditioned cues (Wallace et al., 1992). Addition, unilateral microinjections of morphine into the hippocampus were found to produce CPP (Corrigall and Linseman, 1988). Taken together, although the mesolimbic pathway is an important system that mediates the effects of drugs of abuse, the structures in the limbic system may also alter the ability of drugs of abuse to stimulate CPP.

4.6.2 LFPs in the striatum and the NAc induced by repeated administration and withdrawal of morphine

In the present study, it was observed that repeated morphine administration altered brain oscillations in the striatum and the NAc. The present results demonstrated the initial morphine exposure on the first day appeared to increase power in alpha range in both regions. Though, it was not significant in both regions. Therefore, repeated morphine administration resulted in decreased alpha power in both brain regions. Moreover, it was not significant in NAc. These data suggested that the striatum and the NAc involved with brain adaptation to repeated morphine. The most dramatically changes were seen following morphine withdrawal. Great increases in alpha power were observed in the NAc. However, the mechanisms underlying opiate dependence are not fully understood. Previously, studies in the locus coeruleus (LC), the major noradrenergic nucleus in the brain, has served as a useful model of opiate action (us reviewed by Nestler and Aghajanian, 1997). During a withdraw state induced by application of opiate antagonist, LC neurons in brain slices obtained from morphine-dependent animals exhibited spontaneous firing rates more than two fold higher than that of LC neurons in brain slices from normal animals (Kogan et al., 1992). Increasing in basal firing rate, possibly mediated by an up-regulation of the intrinsic cyclic adenosine 5'-monophosphate (cAMP) pathway, contributes to the hyperactivity of the LC during opiate withdrawal. The upregulated cAMP system in the LC can be viewed as a compensatory or homeostatic response of

LC neurons. Several evidence appeared to support that chronic morphine significantly up-regulates level of cAMP through the activity of adenylyl cyclase (Matsuoka et al., 1994). Blocking the cAMP pathway significantly reduces withdrawal (Bie et al., 2005). Elevating cAMP levels excite LC neurons via the activation of cAMP-dependent protein kinase and subsequent activation of the nonspecific cation channel (Wang and Aghajanian, 1990). In fact, the spontaneous firing rate of LC neurons requires an active cAMP system and the opening of the nonspecific cation channel (Alreja and Aghajanian, 1991). In addition, protein kinase phosphorylation and activation of cAMP response element– binding protein (CREB) stimulates the expression of a family of genes encoding transcription factors referred to as immediate-early genes, for example, c-fos, c-jun, and zif268 (us reviewed by Nestler, 1992). Consistent with these observations in the LC is the more general observation that mutant mice deficient in CREB show attenuated opiate withdrawal (Maldonado et al., 1996). Taken together, these results provide strong evidence to support the view that the opiate-induced upregulation of the cAMP system represents one mechanism by which opiates produce addictive changes in LC neurons.

Functional role of the NAc remains poorly understood. One target of the mesolimbic dopamine system is believed to play a role in motivational states and is implicated in the reinforcing actions of most drugs of abuse (Koob, 1992b; Koob and Le Moal, 1997). It was found that chronic, but not acute, administration of morphine also increases levels of adenylate cyclase and cAMP-dependent protein kinase in the NAc (Terwilliger et al., 1991). However, regulation intracellular messenger proteins have not been observed the striatum, one target of nigrostriatal dopamine system. Thus, changing of LFP power in alpha range in the striatum and the NAc may imply firing activity of neurons during tolerance/dependent and withdrawal state. Previously, in response to acute injection of morphine, dopamine cells in pars compacta of the SN and VTA increased firing rates slowly and steadily (Nowycky et al., 1978). Therefore, the striatum during repeated morphine exposures decreased alpha power might reflect compensatory or homeostatic response of neurons. Additionally, the NAc during withdrawal increase in alpha power appeared

to be correlated with increase in basal firing rate mediated by an up-regulation of cAMP pathway during opiate withdrawal period.

In conclusion, the effects of repeated morphine administration and withdrawal on LFP were seen in the striatum and the NAc. Repeated exposures to morphine were found to change normal oscillatory patterns in the striatum whereas morphine withdrawal changed oscillatory patterns in the NAc only. Changes in oscillatory patterns during repeated administration and withdrawal period of morphine were found to be correlated with firing rate of neurons in each states of drug addiction as shown in previous reports. However, these findings suggested that the NAc, one target of the mesolimbic dopamine system, is a critical brain reward regions, found to be important for drug addiction mechanism.

CHAPTER 5

CONCLUSION

Among various experimental protocols used to measure drug reward and drug addiction, CPP has been one of the most popular methods. Based on modern and traditional theoretical formulations of Pavlovian conditioning, CPP appears to reflect a preference for a context due to the contiguous association between the context and a drug stimulus. There are advantages of CPP. This method allows for simultaneous determination of CPP and locomotor activity. It is sensitive to both reward and aversion. However, these apparatus might not be practical for the question in probing the neural circuits involved in drug addiction. It is unable to distinguish the identity of each addictive drug types. To address these limitations, the functional biomarkers that can be more directly related to electrical brain response during acute, chronic and withdrawal phases of morphine administration were explored. These brain oscillation biomarkers may be useful for understanding alteration neural circuits of morphine in brain areas involved in reward and addiction. Ultimately, it may be an ideal method to identify brain oscillation biomarkers of morphine.

In this study, signal processing tool was employed to investigate neural networks associated with acute, chronic and withdrawal effects of morphine. The present findings demonstrated neural signaling of animal receiving acute morphine. LFP analysis indicated the increased low and high gamma oscillations in reward related brain areas including the striatum and the NAc. However, high gamma oscillation in the striatum was positively correlated locomotor activity. Additionally, it was observed that repeated morphine administration significantly decreased alpha oscillation in the striatum. Alpha oscillation in the NAc was also decreased during withdrawal morphine. Taken together, the LFP oscillation patterns were likely to present the characteristics of morphine CNS action. Therefore, for findings from this study may be applied possible detection of drug effects in addicted patients. Though, electrode placement are different as scalp electrodes used in patients might pick up

different form of electrical signals. However, additional works are needed in order to prove whether LFP patterns in this study are distinguished from that induced by other drugs of addiction.

For further studies, LFP recording in combination with the CPP paradigm might be performed in addition with microdialysis technique for morphine study. Microdialysis probes are used to collect extracellular fluid in the brain areas of interest for the analysis of neurotransmitters or their metabolites released later on. This would allow for additional investigation of the brain function in terms of neurotransmission of the brain circuits during LFP recording in the CPP apparatus. It is interesting to examine the correlation between electrical activity, neurotransmitter release and behavior induced by either acute, chronic or withdrawal of morphine.

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APPENDICES

Appendix A

Original article

Low gamma wave oscillations in the striatum of mice following morphine administration

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ORIGINAL PAPER#

Low gamma wave oscillations in the striatum of mice following morphine administration

Chayaporn Reakkamnuan · Siriphun Hiranyachattada · Ekkasit Kumarnsit

Abstract Functional role of the striatum in motor control has been widely studied. In addition, its involvement in reward function as a brain area in the dopamine system has also been mentioned. However, neural signaling in the striatum in response to consumption of emotional enhancing substances remained to be explored. This study aimed to investigate local field potential (LFP) of the striatum following morphine administration. Male Swiss albino mice implanted with electrode into the striatum were given an intraperitoneal injection of either saline or morphine (5 or 15 mg/kg). LFP and locomotor activity of individual animals were simultaneously recorded in the recording chamber following the administration. The inspection of LFP tracings revealed the increase in fast wave induced by morphine particularly at a high dose. Statistical analyses were performed using a one way ANOVA followed by Tukey *post hoc* test. Frequency analysis using Fast Fourier transform also confirmed a significant elevation of low gamma (30-44.9 Hz) activity. When analyzed in time domain, significant increase in low gamma power was observed from the 15th to 65th min following 15 mg/kg morphine treatment. Moreover, morphine treatment also exhibited a stimulating effect on locomotor speed. However, regression analyses revealed no significant correlation between low gamma power and locomotor speed. In summary, this study demonstrated the increase in low gamma oscillation in the striatum and this effect was not associated with locomotor activity of animals. Thus, it is possible that low gamma oscillation induced by morphine treatment is related with the reward function.

Keywords Striatum · Local field potential · Low gamma wave · Morphine

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Introduction

Drugs abuse are naturally rewarding which is the reason why they are self-administered by laboratory animals or consumed by humans [1]. In 1954, the brain was demonstrated to have specialized centers for reward functions [2]. The confirmation was made when these brain regions were electrically stimulated and highly rewarding response was obviously produced. In term of mechanism, the midbrain dopaminergic system was particularly sensitive to electrical brain self-stimulation, the operant conditioning method used to determine rewarding effects [for review see 3].

Dopamine is a neurotransmitter important for the rewarding effects of drugs abuse. The dopamine circuits have been extensively studied for neuronal networks of reward and addiction. Most of them focused on the role of the mesolimbic and mesocortical dopamine pathways. These pathways consist of dopamine cells in the ventral tegmental area (VTA) projecting to the nucleus accumbens (NAc) and the frontal cortex respectively. Moreover, the other dopamine system has been studied in Parkinson's disease topics that investigate the nigrostriatal dopamine system with dopamine cells in substantia nigra (SN) projecting into the dorsal striatum. These are two separate dopamine systems with different brain areas.

Currently, a new challenge has been focused on the hypothesis whether both systems participate in reward function and addiction [4]. Anatomically, there is no clear boundaries that completely separate these two midbrain dopamine systems [5, 4]. In addition, tracing study demonstrated that the SN and VTA dopamine cells have overlapping, not distinct, projection fields [6]. Ultimately, brain stimulation has been applied to study the mapping of reward-related circuitry in the brain by using direct electrical stimulation to certain brain regions. The population of midbrain dopamine neurons was seen as a final common pathway for the rewarding effect of the medial forebrain bundle (MFB) stimulation [4].

However reward sites are found both in the SN and the VTA [7]. Movable electrode mapping studies also indicated reward related brain sites within the areas of the dopaminergic cell body regions of the SN and the VTA [8]. Previously, the substantia nigra pars compacta (SNc) was demonstrated to produce dopamine to innervate the dorsal striatum, the brain area involved in motor and reward processes [9]. Taken together, the nigrostriatal dopamine system has been found to possess similar properties to that of the mesolimbic dopamine system in participation of reward function and addiction [4]. Until recently, no direct pattern of electrical brain wave in the striatum has been explored in rewarding events.

This study aimed to investigate local field potentials (LFPs) of the dorsal striatum, the brain area that receives dopaminergic inputs from the SN during morphine administration. Male mice were used for electrode implantation into the striatum. Following morphine treatment, LFPs were recorded. Fast Fourier transform is used for the analysis of frequency spectrum. Changes in some frequency ranges would reflect the activity of the nigrostriatal pathway during morphine administration.

Materials and Methods

Experiments were performed using 3 groups (n = 6-9) of adult male Swiss albino mice (approximately 35 g at the start of the experiment) from Southern Laboratory Animal Facility of Prince of Songkla University (PSU), (Songkhla, Thailand). Animals were housed in standard environmental conditions (24 ± 1 °C and 12 hr light/dark cycle). They had freely access to standard commercial food pellets and filtered tap water. The experimental protocols for care and use of the experimental animals in the present study were approved and guided by the Animals Ethical Committee of the PSU.

For surgical procedure animals underwent stereotaxic implantation of electrode for local field potential recording. Surgery was performed under ketamine/ xylazine (150/15 mg/kg) by intramuscular (i.m.) injection. Therefore, animal's head was fixed with stereotaxic frame through ear pieces as described previously (Fig. 1 a-e) [10]. Briefly, the scalp was shaved and swabbed with betadine. After lidocaine (20 mg/ml) was injected subcutaneously, a midline incision was made at on the scalp. The electrodes were stereotaxically implanted overlying the left striatum area (AP: +1.1 mm, ML: 1.5 mm, DV: 3.5 mm) using bregma as the landmark and the cerebellum (AP: -6.5 mm, DV: 2 mm) as a reference and ground electrode. Additional holes were drilled for stainless steel anchor screws. All the electrodes were linked to a female connector fixed to the skull by dental cement. After surgery, animals were placed in a clean cage with a heating pad and monitored until ambulatory behavior was observed. Antibiotic (100 mg/kg ampicillin) was applied intramuscularly for 3 days to prevent infection. They were allowed to fully recover for at least 7-10 days before the start of the experiment.

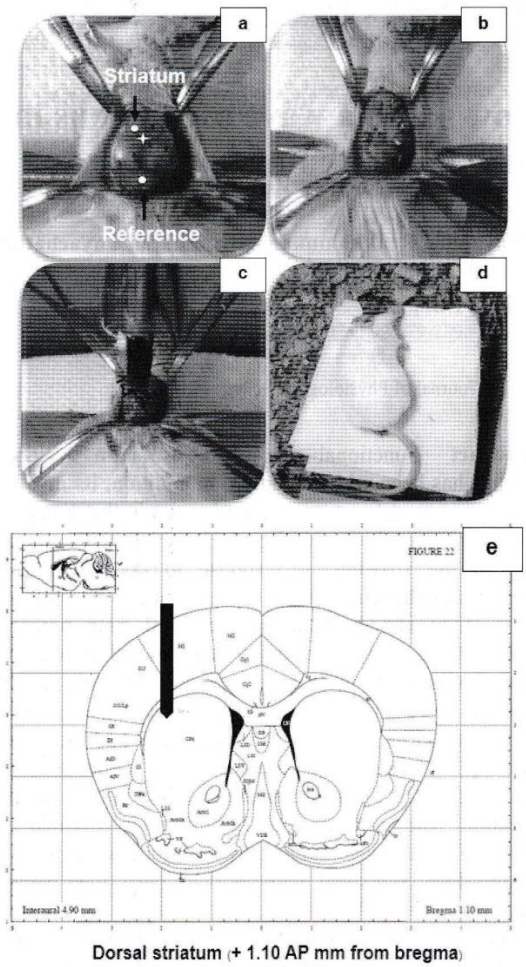

Fig. 1 Electrode implantation. (**a**) Striatum location of electrode implantation on the skull. Cross mark indicates bregma. (**b**) Stainless steel anchor screws were fixed on the skull. (**c**) Intracranial electrodes were implanted into the striatum and cerebellum (reference). (**d**) Dental acrylic was used to secure and fix all electrodes on the mouse skull. (**e**) Coronal mouse brain section of the striatum shows a schematic drawing of silver wired electrode tip placement for LFP recording.

Experimental procedure and local field potential (LFP) recording before LFP recording in response to acute morphine administration, the animals were habituated with the recording condition in a chamber for 4 hrs per day for 3 consecutive days. Then, baseline recording for one hour was required before intraperitoneal injection of either saline or morphine (5 or 15 mg/kg). Post-drug recording was performed for 3 hrs following the

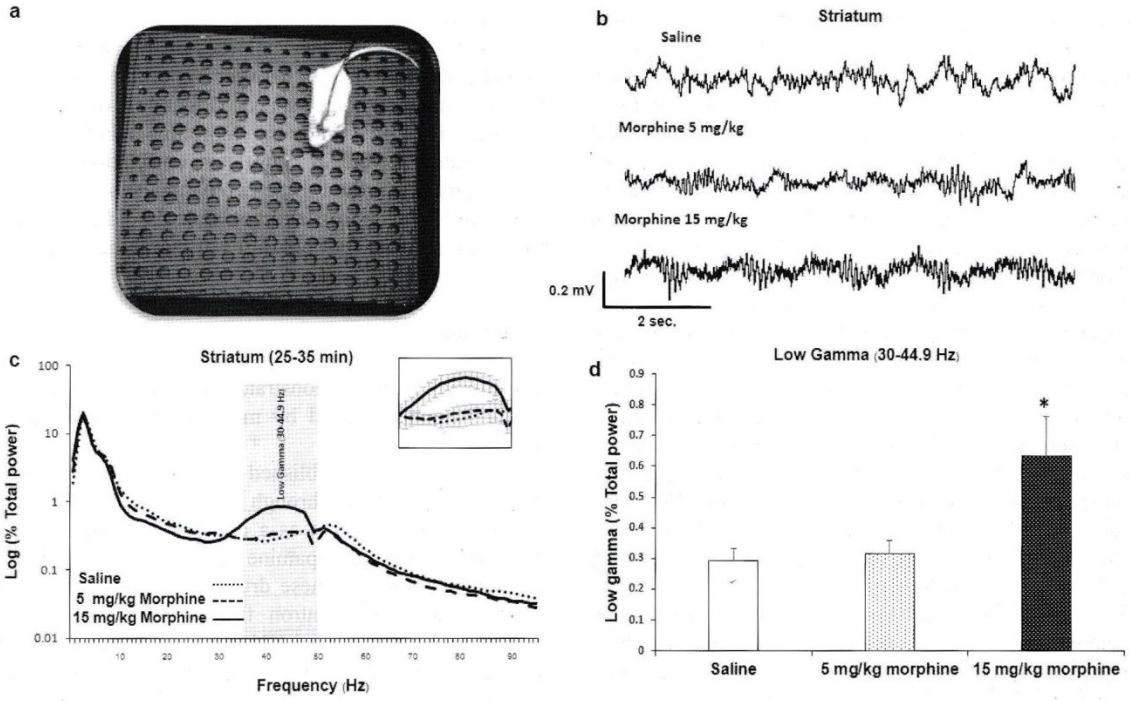

Fig. 2 Processes of LFP recording and analysis following acute morphine administration. (**a**) Individual animals were allowed to explore in the recording chamber. (**b**) Raw striatum LFP signals recorded from representative mice that received saline, 5 mg/kg morphine and 15 mg/kg morphine were displayed in time-domain. (**c**) Power spectrums of striatum LFP are expressed in frequency domain. (**d**) Averaged percent total power of low gamma range are expressed as mean ± S.E.M. * $P < 0.05$ compared with the saline control group (one-way ANOVA followed by Tukey's *post hoc* test).

injection. LFP signals were amplified with low-pass 200 Hz, high-pass 1 Hz and digitized at 2 kHz by a PowerLab 16/35 system (AD Instruments, Castle Hill, NSW, Australia) with 16-bit A/D. Data were stored in a PC through the LabChart 7 program software. 50 Hz notch filtering was applied to remove the noise from power line artifacts. All LFP signals were processed through 1–200 Hz band-pass digital filter (raw filtered signal). Locomotor activity of animals was recorded by using a video camera mounted on the top of the recording chamber. The recording method and analysis of locomotor speed were done as previously described [10].

For spectral power analysis, power spectral density (PSD) was generated by LabChart 7 software using Hanning window cosine with 50% window overlapping and 0.976 Hz frequency resolution. Then, the PSD in each frequency bin was expressed as the percentage of total power (1-100 Hz). The average spectral power were constructed in discrete frequency bands of each group and expressed in frequency domain. In this study, power spectrum in the striatum LFP was divided into slow wave (1-4 Hz), theta (4-8 Hz), alpha (9.7-12 Hz), beta1 (13.6-18 Hz), beta2 (19.5-29.3 Hz), low gamma (30-44.9 Hz) and high gamma (60.5–100 Hz).

All data were averaged and expressed as mean ± Standard Error of Mean (S.E.M.). Differences between the saline and morphine (5 mg/kg or 15 mg/kg) were analyzed by using one-way analysis of variance (ANOVA) followed by multiple comparisons using Tukey's *post hoc* test to indicate specific points of significance. In addition, linear regression analyses between striatum LFP power and locomotor speed were also analyzed. Levels of significance were set at $P < 0.05$.

Results

Following the administration of saline or morphine (5 and 15 mg/kg), LFP signals from individual mice were continuously recorded for 3 hrs (Fig. 2a). Representative raw LFP tracings of saline, 5 and 15 mg/kg morphine groups were shown (Fig. 2b). By visual inspection, relatively equal slow wave activities of striatum LFPs were seen among groups. However, the slow oscillations appeared to be superimposed with fast wave activity in morphine groups particularly at a 15 mg/kg dose especially during 25 to 35 min. Therefore, frequency

analysis of raw LFPs during a period of 25-35 min was conducted for percent total power in a broad frequency range from 1 to 100 Hz (Fig. 2c). Obviously, morphine treatment (15 mg/kg) appeared to specifically increase power in a range of low gamma oscillation. Statistical analysis also confirmed that significant increase in low gamma (30-44.9 Hz) power was seen in the group of high dose of morphine (Fig. 2d). No significant difference was produced by 5 mg/kg morphine.

Therefore, effects of morphine treatment on low gamma oscillation in the striatum were particularly analyzed in time domain (Fig. 3). Data were converted to percent total power and analyzed every 5 mins. Differences in percent total power of low gamma frequency compared to control levels were determined by using one-way ANOVA followed by Tukey's *post hoc* test. It was found that 15 mg/kg morphine began to produce significant increases from the 15th until 65th min. Peak effect was observed during the 30th min. No significant change in percent total power of low gamma was induced by 5 mg/kg morphine.

For the effects of morphine administration on locomotor activity, the results showed that morphine dose dependently increased averaged speed and travelled distance in comparison to saline control group (Fig. 4a and b). One-way ANOVA revealed that locomotor speed [F (2, 24) = 11.522; $P < 0.001$] and travelled distance [F (2, 24) = 6.868; $P < 0.001$] were significantly increased in 15 mg/kg morphine group. No significant change was observed in 5 mg/kg morphine group for both locomotor parameters.

In addition, regression analyses were performed to evaluate the correlation between striatum low gamma power and locomotor speed (Fig. 4c) or travelled distance (Fig. 4d) following morphine administration. The results showed no significant correlation between low gamma power and these two parameters. Locomotor speed and travelled distance of animals did not predict low gamma power for both doses of morphine.

Discussion

The present study demonstrated low gamma oscillation in the striatum induced by morphine administration in mice.

It has been well established that most addictive drugs produce their effects through activity of the dopamine neurotransmitter system as a common mechanism [11]. Their effects on the dopamine system were dominant as the administration of these drugs was found to increase midbrain dopamine neuron firing [12] and dopamine release preferentially in the NAc [13]. In contrast, drugs with aversive properties were demonstrated to reduce dopamine release in the NAc [13]. In terms of mechanism, the opiates have been proposed to activate dopamine cells via non-dopamine cells, through μ-opiate receptors located on GABAergic midbrain interneurons that have inhibitory tone on dopamine cell firing [14]. Activation of these inhibitory $G_{\alpha i}$-coupled μ-opiate

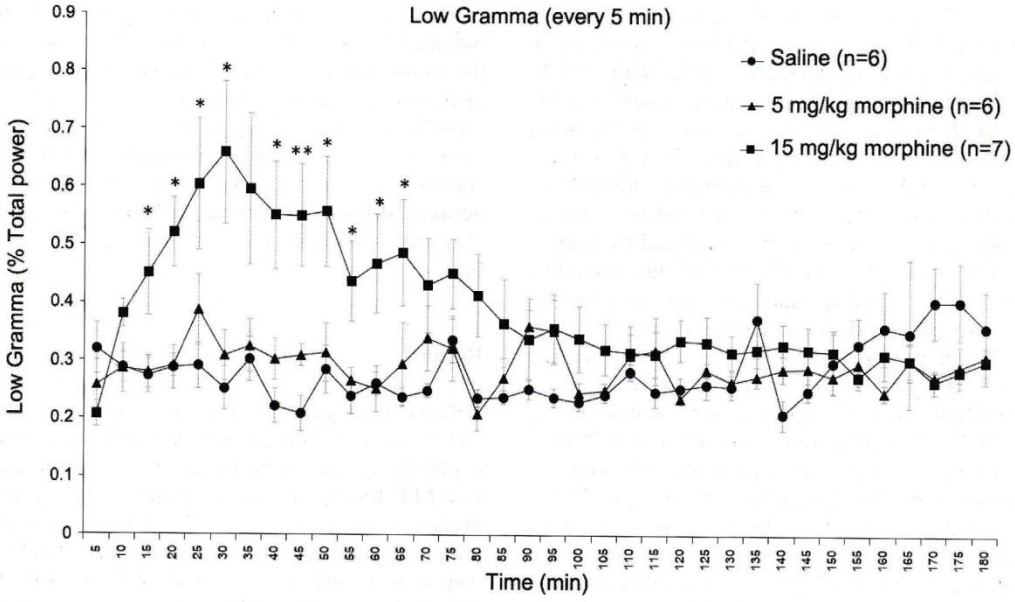

Fig. 3 The average percent total power of low gamma range were analyzed every 5 min period after injection of morphine (5 and 15 mg/kg) or saline. Data were compared with that of saline control group using one-way ANOVA followed by Tukey's *post hoc* test. *, **: $P < 0.05$ and $P < 0.01$, respectively.

J Physiol Sci (2015) 65 Suppl 2: S-11 – S-16

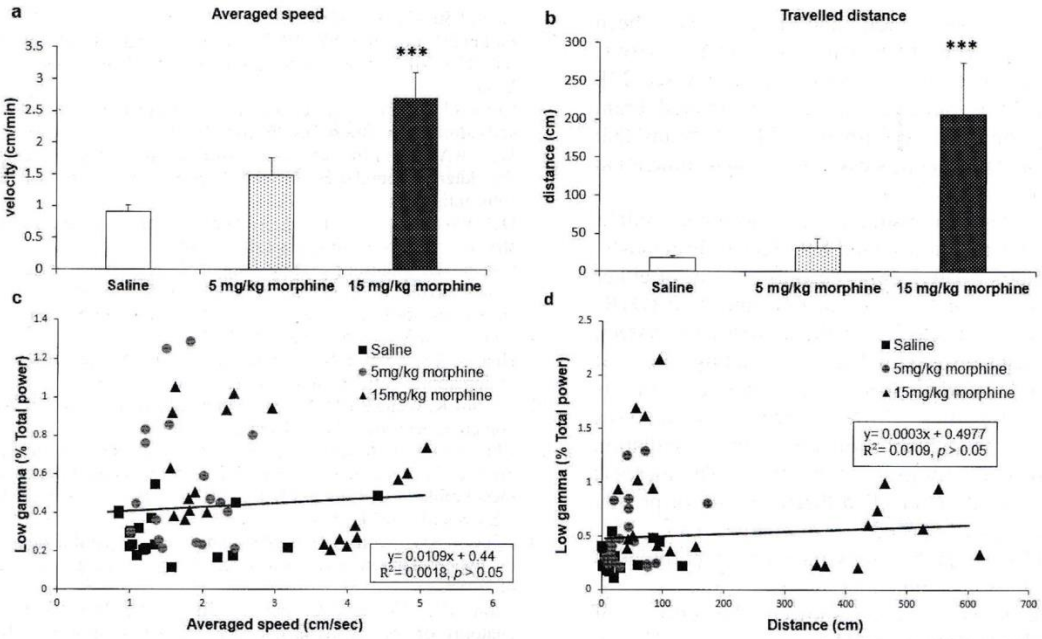

Fig. 4 Morphine induced averaged speed and travelled distance. (**a**) Averaged speed and (**b**) travelled distance during morphine administration expressed as mean ± S.E.M. (**c**) Regression analyses between striatum low gamma oscillation and averaged speed and (**d**) travelled distance during morphine administration. Data were analyzed using one-way ANOVA followed by Tukey's *post hoc* test. ***: $P < 0.001$ compared with the saline control group.

receptors was found to withdraw the GABAergic tone from midbrain dopamine neurons which, in turn, resulted in increasing firing rate and the amount of dopamine released in the NAc [11]. Moreover, additional research findings also demonstrated that morphine increased cell firing levels in both 2 origin dopaminergic areas, the VTA and the SNc [12] and extracellular dopamine concentrations in 2 terminal dopaminergic areas, the NAc and the striatum [13].

The striatum is among main components of the basal ganglia complex. Its principal functions are primarily related to motor control. The nigrostriatal dopamine pathway (with dopamine cells locating in the SN projecting their axons to the striatum) is one of neural circuits that also has important roles in movement [15]. Dopamine is produced by cells in the pars compacta of SN. Nigrostriatal axon terminals release dopamine into the striatum to produce an excitatory effect upon cells in the striatum [15]. The deficits of dopamine pathway are associated with movement disorders such as Parkinson's disease [16]. Basically, Parkinson patients have considerable difficulties in initiation and termination of movement. Later, the involvement of this pathway in reward processes has been studied [17]. Therefore, it has been discussed that the nigrostriatal dopamine pathway also plays a significant role in reward in addition to that of the mesolimbic and mesocortical dopamine pathways [for review see 4].

The present study clearly demonstrated that a significant increase in low gamma power was observed from the 15th to 65th min following 15 mg/kg morphine treatment. It has been well established for the rewarding properties of morphine [11, 18]. Most of classical studies of reward function have focused on the activity of the ventral striatum, also known as the NAc [for review see 3]. Previously, the study of local field potentials in the ventral striatum demonstrated reward-associated gamma oscillations [19]. On the other hand, gamma oscillations in the dorsal striatum were partially correlated with movement initiation [20]. However, the stimulation of the SN, the brain areas that projects neural pathway mainly to the dorsal striatum, also produced rewarding effect [8]. Previously, lesions of the dorsal striatum were found to reduce reward response to either cocaine or morphine [21]. In particular, brain imaging study using positron emission tomography (PET) in human cocaine addicts demonstrated an increase in dopamine release within the dorsal striatum in response to cocaine associated cues [22]. Taken together, these findings suggest some degree of involvement of the dorsal striatum in drug reward and addiction. Therefore, it is likely that the enhanced gamma oscillation seen in the present study might be associated with reward induced by morphine treatment. Until recently, no direct link

between gamma oscillation and reward has been established. In general, the increase in gamma activity is involved in information processing [for review see 23]. For example, it is dominant in learning related brain areas during cognitive performance [24]. It means that information signaling is processed to mediate functional roles of the brain areas.

Following the administration of morphine, either reward or motor functions could be affected. Previously, reduced locomotor activity was seen as a result of either dopamine D1 [25] or D2 [26] receptor knockout (D1R-KO or D2R-KO respectively) in the nigrostriatal system. The present data also exhibited a stimulating effect of morphine on locomotor speed that would confirm its psychomotor properties. However, regression analyses confirmed that the induction of low gamma oscillation was not correlated with locomotor activity. The increase in locomotor speed or travelled distance did not predict low gamma power induced by morphine. Thus, it is possible that low gamma activity induced by morphine is associated with reward function.

In conclusion, this study showed the effects of morphine administration on LFP oscillation in the striatum and locomotor activity in mice. The increase in low gamma activity was not correlated with motor function. Therefore, it was proposed to reflect rewarding process of morphine. Altogether, these findings emphasized the involvement of the striatum in reward function and demonstrated a highlight of low gamma oscillation in response to morphine treatment.

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

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

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Appendix B

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July 21 , 2015

This is to certify that the research project entitled "EEG biomarkers of morphine reward in mouse brain" 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.

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List of Publication

Reakkamnuan, C., Hiranyachattada, S. and Kumarnsit, E. 2015. Low gamma wave oscillations in the striatum of mice following morphine administration. *The Journal of Physiological Sciences*. 65 (Supplement2): 11-16.

List of Poster presentation

Reakkamnuan, C., Hiranyachattada, S. and Kumarnsit, E. 2015. Low gamma wave oscillations in the striatum of mice following morphine administration. *The Journal of Physiological Sciences*: The 8 th Congress Federation of the Asian and Oceanian Physiological Societies, November 22-25, 2015, Bangkok, Thailand.