Nucleus accumbens local field potential power spectrums, phase-amplitude couplings and coherences following morphine treatment

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In the past decade, neural processing has been extensively studied in cognitive neuroscience. However, neural signaling in the nucleus accumbens (NAc) that might clarify reward process remained to be investigated. Male Swiss albino ICR mice implanted with intracranial electrodes into the NAc and the ventral tegmental area (VTA) were used for morphine administration and local field potential (LFP) recording. One-way ANOVA revealed significant increases in low (30.3-44.9 Hz) and high (60.5-95.7 Hz) gamma powers in the NAc following morphine administration (5 and 15 mg/kg, i.p.). These gamma activities oscillated independently with different time-course responses. Locomotor activity was also significantly increased by morphine administration. Regression analyses revealed that high gamma activity induced by morphine was positively correlated with distance travelled by animals. Low and high gamma powers were completely abolished by injection of naloxone, a non-specific opiate antagonist. Analysis of phase-amplitude coupling confirmed that slow oscillations at 1-4 Hz (delta) and 4-8 Hz (theta) for phase were found to significantly increase modulation index of broad (30.27-80.77 Hz) and narrow (59.48-70.34 Hz) frequency ranges for amplitude, respectively. Moreover, significant increases in coherence values between the NAc and the VTA during 30-40 min following morphine administration were seen for 22.46-44.90 Hz frequency range. Altogether, this study demonstrated changes of LFP oscillations in the NAc with low and high gamma activities, delta- and theta-gamma couplings and interplay with VTA in response to morphine administration. These findings represent neural signaling in the mesolimbic dopamine pathway that might process reward function.

Key words: morphine, reward, nucleus accumbens, local field potential, power spectrum, theta-gamma coupling

INTRODUCTION

Biological mechanisms in the brain that mediates reward have been studied for better understanding of pleasure. In neuroscience term, reward has been focused on functions of neural structures that are critically involved in mediating the effects of pleasurable stimuli. Moreover, drugs of addiction have been extensively used in animal research according to their inducing effects on pleasurable state which describes a feeling of reward (Van Der Kooy et al. 1982, McIntyre et al. 1998). Drugs of abuse are very powerful reinforcers that motivate high rates of operant responding. A midbrain-forebrain circuit particularly with its focus in the nucleus accumbens (NAc) has been involved in reward function (Koob 1992b). Several findings demonstrated that dopamine is a CNS neurotransmitter important for the rewarding effects of drug abuse (Fibiger et al. 1987). The dopamine circuits have been extensively studied for neuronal networks of reward and addiction (Yokel and Wise 1975, Koob 1992a, Volkow et al. 2011). Among these circuits, the mesolimbic pathways consist of dopamine cells in the ventral tegmental area (VTA) projecting to the NAc and the frontal cortex. Most of classical addictive drugs including heroin (Xi et al. 1998), amphetamine (Selling and Clark 2003), cocaine (Uchimura and North 1990), ethanol (Yim and Gonzales 2000) or even pseudoephedrine (Kumarnsit et al. 1999) have been demonstrated to act on this brain system. It is also noted that various psychoactive drugs were classified as addictive substances mostly by using the same techniques including self-administration or place preference paradigms. These findings suggested that substances with positive reinforcing effects trigger some common CNS mechanisms and produce similar behavioral consequences. However, these individual drugs have also been found to exhibit unique characteristics. They might also exert CNS actions through distinctive pharmacokinet-
ics to produce specific patterns of tolerance or withdrawal symptoms.

Morphine is an opioid drug and the main psychoactive chemical in opium. From the beginning, therapeutic use of morphine and other opiates has been known for analgesia (Abbott et al. 1982). It acts directly on the CNS to decrease the feeling of pain. However, morphine also has a high potential for addiction and abuse (Wikler 1948). Abrupt cessation or reduction of the dose after long term use appeared to induce withdrawal symptoms (Maldonado et al. 1992). In particular, structural alterations in the mesolimbic dopamine pathway induced by chronic morphine treatment were believed to underlie addiction (Sklair-Tavron et al. 1996). Altogether, the central role of the opioid system in drug-addictive processes have been supported by numerous studies using opioid antagonists such as naltrexone or naloxone in experimental animals (Ettenberg et al. 1982, Samson and Doyle 1985, Kornet et al. 1991, Pothos et al. 1991, Negus et al. 1993).

However, neural processing that mediate reward function induced by morphine remains to be characterized. In the past decade, neural signaling has been extensively focused particularly in cognitive neuroscience to monitor dynamic responses of the CNS in some particular conditions. Therefore, recordings of local field potential (LFP) in discrete brain regions were designed to investigate subtle mechanisms of morphine in the brain. In this study, intracranial electrodes were implanted into the NAc, a reward related brain region, of mice for LFP recording. Fast Fourier Transform was used to analyze frequency components of the LFP signals. Locomotor activity of animals was monitored and tested for its correlation with LFP oscillatory patterns following morphine administration.

**MATERIALS AND METHODS**

**Animals**

Male Swiss albino ICR mice (7-8 weeks old) provided by Southern Laboratory Animal Facility of Prince of Songkla University, (Songkhla, Thailand) were used. This study was carried out in accordance with guidelines of the European Science Foundation (Use of Animals in Research 2001) and International Committee on Laboratory Animal Science, ICLAS (2004). 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 Prince of Songkla University (MOE 0521.11/840). All efforts were made to minimize animal suffering and to reduce the number of animals used. In total, 43 mice were used for all experiments.

![Experimental procedure testing the effects of morphine and naloxone administrations on spontaneous local field potential (LFP) signal and locomotor activity. Altogether, animals received intracranial electrode implantation, habituation sessions and recording schedule. They were acclimatized with the recording system before the testing day.](image-url)
Surgery for intracranial electrode implantation

Experimental protocol started with electrode implantation into the brain of animals and followed with acclimatization to minimize stress during the test and the finally the start of LFP recording (Fig. 1). Surgery process was performed for stereotaxic implantation of electrodes into the NAc and the VTA for LFP recording. A mixture of 150 mg/kg ketamine (Calypsol, Gedeon Richter Ltd., Hungary) and 15 mg/kg xylazine (Xylavet, Thai Maji Pharmaceutical co., Ltd., Thailand) as a cocktail was given to animals by intramuscular (i.m.) injection. The surgery started after animals were deeply anesthetized. Animal’s head was fixed with the stereotaxic frame through the ear pieces. Therefore, the scalp was shaved and swabbed with betadine. After lidocaine (20 mg/ml) was injected subcutaneously, an incision was made at a midline of the scalp. Silver wire electrodes (A-M system, Sequim, WA, USA) with bare diameter of 0.008” (Coated-0.011”) were stereotaxically implanted into the left NAc (AP: + 0.7 mm, ML: 1.3 mm, DV: 4.7 mm) and VTA (AP: -3.0 mm, ML: 1.0 mm, DV: 5 mm) using bregma as the landmark and the cerebellum (Midline, AP: -6.5, DV: 2) as a reference and for ground electrode. The implantation to the striatum (AP: +1.1 mm, ML: 1.5 mm, DV: 3.5 mm) was also performed as a landmark of movement-related brain area. The accuracy of intracranial electrode placements was thoroughly checked after the experiment was completed. Animal’s brains were coronally cut through the brain areas of LFP study. The brain sections were visualized to confirm locations of lesion made by electrode placement using mouse brain atlas. All the electrodes were linked to a female connector fixed to the skull by dental cement. After surgery, animals were allowed for approximately 10 days to recover fully. The antibiotic ampicillin (General Drug House Co., Ltd., Thailand) (100 mg/kg ampicillin) was applied (100 mg/kg, i.m.) for 3 days to prevent infection.

The experimental setup and LFP recording

Before LFP recording in response to acute morphine administration, animals were habituated with the recording condition in a chamber for 210 mins per day for 3 consecutive days. On testing day, baseline recording for 30 mins was required as pre-drug recording before intraperitoneal injection of either saline or morphine (5 or 15 mg/kg). Post-drug recording was performed for 180 mins following the injection. Moreover, the effects of 20 mg/kg naloxone administration (opioid antagonist) on LFP induced by morphine were tested. Thirty minutes following morphine treatment, animals were intraperitoneally injected with 20 mg/kg naloxone. Therefore, LFP signal was continuously recorded for 150 mins (Fig. 1). 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 noises 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. For the analysis, the images of moving animal were continuously transferred to a computer for data processing. The custom made computer software (visual C++) was used for analysis of animal move.

Fig. 2. Raw LFP signals and power spectra in the nucleus accumbens (NAc) following morphine administration. Representative LFPs from mice that received saline, 5 mg/kg morphine and 15 mg/kg morphine are displayed in time-domain (A). Power spectra of NAc LFP are also expressed in frequency domain from 0 – 100 Hz (B).
LFP data analysis

Spectral frequencies of LFP signals were analyzed. Power spectral density (PSD) was generated by LabChart 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 was constructed in discrete frequency bands of each group and expressed in frequency domain. In this study, broad power spectrum of LFP in the NAc was particularly focused for low gamma (30–44.9 Hz) and high gamma (60.5–100 Hz) ranges.

In general, electrical brain activity could be related with sensorimotor functions. From previous report, morphine administration was found to increase in both gamma sub-frequency ranges and locomotor activity. Therefore, correlations between locomotor activity and both gamma powers after morphine administration were examined as described previously (Cheaha et al. 2015, Reakkamnuan et al. 2015). It is necessary to determine whether LFP oscillation is correlated with locomotor activity or not. Therefore, regression analyses were also performed between locomotor activity and NAc oscillation following saline, 5 mg/kg morphine and 15 mg/kg morphine administration. Signals were also taken from the striatum, the brain region that plays a critical role in sensorimotor function, for positive correlation between gamma power and locomotor activity.

Cross spectrum analysis between LFP oscillations in the NAc and the VTA (coherence)

The coherence spectrum is a measure for the interdependence of LFP signals from 2 sources in the frequency domain. The magnitude square coherences between two paired brain areas (VTA - NAc) after receiving saline, 5 mg/kg morphine and 15 mg/kg morphine administration were calculated by using Brainstorm3 software (Tadel et al. 2011) and expressed as coherence spectrogram. Coherence values were averaged for selected frequency bands and compared with that of saline group.

Phase-amplitude coupling (PAC) analysis

The phase-amplitude coupling analysis was performed by using Brainstorm3 software. The modulation index (MI) measures the coupling strength between 2 discrete frequency ranges of interest: a phase-modulating and an amplitude-modulated frequencies. The raw digitized signals were filtered for slow-phase modulating (1–4 and 4–8 Hz) and fast-amplitude modulated (approx. 30–100 Hz) frequency ranges. The interplay between these rhythmic oscillations was investigated in term of slow frequency (delta and theta) phase modulation of fast frequency (gamma) amplitude and expressed as modulation index. The data of each group were calculated and expressed as the PAC map or comodulograms and averaged MI values.

Statistical analysis

All data were averaged and expressed as mean ± Standard Error of the Mean (S.E.M.). The effects of morphine administration on LFP power in time domain, locomotor activity, coherence values and phase-amplitude coupling were analyzed by repeated measure one-way ANOVA followed by multiple comparisons with Tukey’s post hoc test to indicate specific points of significance. In addition, linear regression analyses between LFP power and locomotor activity were also analyzed. Differences were considered statistically significant at p-value < 0.05.

Fig. 3. Averaged percent total power of low gamma (A) and high gamma (B) frequency ranges following morphine administration. Data were analyzed every 5 min period before and 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.
RESULTS

LFP signals and power spectrums following morphine administration

Following morphine administration, LFP signals in the NAc were recorded and representative raw LFP tracings were displayed. The overview of the signals obviously detected the oscillatory character induced by morphine (Fig. 2A). Mainly, morphine injection appeared to increase fast brain wave particularly at high dose (15 mg/kg). Relatively more fast waves induced by morphine were superimposed in raw LFP signals. Morphine appeared to increase low (30.3–44.9 Hz) and high (60.5–95.7 Hz) gamma power (Fig. 2B). Dose-dependent effect of morphine seemed to be predominant on low gamma activity compared to that of high gamma frequency range.

Time-course effects of morphine administration on low and high gamma powers in the NAc

Gamma activities (low and high sub-frequency ranges) were particularly monitored every 5 min from baseline levels and following morphine administration. After morphine administration rapid increase in low gamma power was observed (Fig. 3A). Particularly, significant increases produced by a high dose of morphine (15 mg/kg) were obviously seen from the 15th min and saturated at around the 35th min after morphine administration. Low gamma activity was maintained at high level for almost 40 min. Therefore, it was gradually declined to control level at 150th min. Significant increases in low gamma power induced by low dose of morphine (5 mg/kg) were seen in lower magnitude and narrower time period. In contrast, the increased high gamma activity was found to be a slower response to 15 mg/kg morphine adminis-
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. There was no significant difference induced by 5 mg/kg morphine. Altogether, these 2 gamma oscillations were generated independently of each other.

**Effects of morphine administration on locomotor activity**

Locomotor activity was analyzed in terms of distance travelled made by animals following the administration (Fig. 4A). Morphine administration was found to have significant effects on averaged distance \( F_{2,17} = 10.529, P < 0.01 \). Multiple comparisons also indicated significant effect of morphine treatment at high dose (15 mg/kg morphine).

**Correlations between distance travelled and gamma powers following morphine administration**

Data were calculated from signals recorded in the NAc during 30–40 min and 95–110 min period after morphine treatment for correlation analysis between low or high gamma powers, respectively. In striatal LFP analyses, a significant correlation between high gamma power and distance travelled was obtained following 15 mg/kg morphine treatment \( R^2 = 0.47, P < 0.05 \) (Fig. 4B). For NAc LFPs, a significant correlation was found for high gamma power \( R^2 = 0.17, P < 0.05 \) (Fig. 4D) but not low gamma power \( R^2 = 0.09, P > 0.05 \) (Fig. 4C). The distance travelled clearly predicted gamma power both in the striatum and nucleus accumbens following morphine treatment at the high dose.

**Effects of naloxone injection on NAc low gamma and high gamma powers induced by morphine administration**

Low gamma and high gamma activities in the NAc induced by morphine treatment were determined whether these activities are dependent on opiate receptors. Thirty minutes after morphine treatment (15 mg/kg), animals were given naloxone injection (20 mg/kg). Signals were continuously recorded and analyzed from baseline activity and following the administrations with morphine and naloxone. Data were shown and analyzed every 5 min in comparison with that of control (saline/saline) and morphine (morphine/saline) groups. The results showed that the immediate response in increase in low gamma power induced by morphine was completely abolished within the first 5 min of data analyzed (Fig. 5A). Naloxone was capable to extinguish the effects of morphine on low gamma power throughout the time course analyzed. Similar effects of naloxone on the slow response in increase in high gamma power induced by morphine were also seen (Fig. 5B). Significant effects of naloxone were prompt and continuous until the end of recording period.

**Phase-amplitude coupling in the NAc following morphine administration**

Slow frequency modulation of gamma activity was analyzed before and after morphine administration (30–40 min). In comodulogram maps, patterns of phase-amplitude couplings were clearly induced by both low and high doses of morphine (Figs 6D and 6F) compared with pre-treatment (Figs 6C and 6E, respectively). These patterns of coupling were not seen in control animals (Figs 6A and 6B). Therefore, data were analyzed in terms of modulation index measured from phase-modulating delta
(1–4 Hz) and theta (4–8 Hz) frequencies. Within the delta phase, amplitude of gamma frequency was significantly increased (Fig. 6I) compared to basal level (Fig. 6G). Broad range of modulated gamma activity (from 30.27–80.77 Hz) was clearly induced by the high dose of morphine. The low dose of morphine was also found to significantly induce delta-gamma coupling with relatively lower modulation index and narrower range of gamma frequency. Moreover, minimal changes in phase-modulating theta (Fig. 6J) compared to basal level (Fig. 6H). Only a 59.84–70.34 Hz frequency range was significantly modulated following morphine administration (high dose). No significant change in theta-gamma coupling was induced by low dose of morphine.

**Coherence between LFPs in the NAc and the VTA**

LFP oscillations in the NAc and the VTA in relation with frequency spectrum were analyzed to examine the interplay between these 2 separate brain areas. Data were analyzed during 30–40 min period following morphine administration. Values of coherence were calculated for 1–100 Hz range. Following morphine administration, coherence values between these 2 areas were significantly increased within broad (22.46–45.90 Hz) and narrow (38.09–42.97 Hz) frequency ranges induced by high and low doses of morphine, respectively (Fig. 7). No significant difference in coherence value was seen at higher frequency range.

**DISCUSSION**

The present results showed that morphine significantly increased locomotor activity in comparison to saline control group. These results are consistent with those of previous studies (Becker et al. 2000, Yoo et al. 2003). In general, drugs of abuse, such as opiates enhance locomotor activity (Kalivas and Duffy 1987). 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). In term of mechanism, some research indicated that dopamine also plays an important role in locomotor activity. Dopamine-deficient mice were unable to produce a normal locomotor response but, with high dose of morphine treatment, dopamine-deficient mice were found to significantly increase locomotor activity (Hnasko et al. 2005). This study suggested that morphine may stimulate locomotor through dopamine neurotransmission. However, there was 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 knock-out (D1R-KO or D2R-KO respectively) in the nigrostriatal system. These previous findings suggested that locomotor activity induced by morphine was mediated by opiate receptors through an indirect dopaminergic mechanism (Zarrindast and Zarghi 1992).

In the present study, morphine administration clearly affected LFP oscillations in the NAc with some modifications of low and high gamma activities 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, 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 $\alpha_i$-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 substantia nigra pars compacta (SNc) (Gysling and Wang 1983) and extracellular dopamine concentrations in 2 terminal dopaminergic areas, the NAc and the striatum (Lübetzki 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). Nigrostriatal dopamine pathway (with dopamine cells located in the substantia nigra (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 pars compacta of the 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.

The present study clearly demonstrated two different patterns of low and high gamma oscillations in the NAc induced following morphine treatment. Low gamma power was increased from the 15th to 150th minute whereas high gamma power was observed from the 80th to 175th min following 15 mg/kg morphine treatment. These findings proposed at least two distinct mechanisms underlying gamma oscillations in this brain area. With signaling analysis, NAc LFPs were found to have separate fast and slow responses to morphine treatment. However, both responses were completely sensitive to naloxone administration. It means that both low and high gamma oscillations in the NAc were solely dependent on opiate receptors. Gamma brain wave can be 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). Our regression analyses confirmed that the induction of high gamma oscillation in the NAc by morphine (15 mg/kg) was significantly correlated with distance travelled by animals. Moreover, numerous classical studies also suggest that the activity of the NAc, also known as the ventral striatum, is associated with the maintenance of reinforcement processes, or the subjective rewarding actions of natural rewards and drugs of abuse (Spanagel and Weiss 1999). Therefore, multiple gamma oscillations might be correlated with various mechanisms of reward function. Previously, it was reported that the low gamma range is more related with reward than 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). Previously, 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 addition, low gamma was completely abolished upon reward receipt while high gamma only showed a transient increase to reward delivery (Berke 2009).
To date, there are still some discrepancies of research findings. The modulation of ventral striatum gamma by reward is still a topic of active research and is not explained by one theory. Anyway, shifts of low and high gamma activities might represent neural processing of reward function in the NAc.

Additionally, the present study clearly demonstrated positive correlations between high gamma oscillation in the striatum and locomotor activity. The striatum receives dopaminergic projections from the SN and is known as the brain region primarily involved with motor function (Marsden 1982). Previously, high gamma band activity in the striatum of human was observed during finger movement (Huo et al. 2010) and in the same area of rats 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). Previously, intracranial self-stimulation were demonstrated to be dependent on ascending fibers of the mesotelencephalic DA projections and dopamine release in several brain regions including the striatum and NAc (Fibiger et al. 1987). In the NAc, dopamine release was also correlated with increased locomotor activity (Munoz-Villegas et al. 2017). Recently, the operant behavioral study confirmed that dopamine release in the nucleus accumbens is a major regulator of behavioral responding particularly at the motivational level to reward (Bergamini et al. 2016). These findings suggested functional link between reward and locomotor system that might be necessary to initiate or enhance movement such as food or drug seeking. Thus, modulation of high gamma oscillatory activities both in the striatum and NAc may be key features of neural processing associated with motor component of reward function.

Interaction between slow frequency phase and gamma power has been measured extensively in learning and memory processes during the past decades. Increase in strength of theta-gamma modulation in the hippocampus of rats was associated with spatial learning (Nishida et al. 2014). Theta-gamma coupling was modulated in particular hippocampal subfields during the spatial task (Bott et al. 2015). Alterations of theta-gamma coupling were seen in a mouse model of Alzheimer’s disease (Goutagny et al. 2013). Similar findings of theta-gamma coupling in association with learning and memory were also observed in human (Park et al. 2011, Park et al. 2013). This type of cross frequency interaction has been found in various brain regions apart from the hippocampus including the inferior occipital gyrus (Sato et al. 2014), the anterior thalamus (Sweeney-Reed et al. 2015). Recently, enhanced theta-fast gamma coupling in the basolateral amygdala was detected during periods of fear (Gail et al. 2004). In the present study, significant increases in gamma power were modulated by both delta and theta phases following morphine administration. According to time course of the induction, it is likely that these phase-amplitude couplings are correlated with reward effects produced by morphine treatment. However, direct correlations are needed to be established to confirm that theta-gamma coupling in the NAc reflects reward function processes.

The present data also demonstrated the increases in coherence value of LFP signals between VTA and the NAc, the origin and terminal areas, respectively, of the mesolimbic dopamine pathway. These findings are consistent with dopamine release in the NAc and reward action following morphine injection (Pothos et al. 1991). Previously, the coherence has been analyzed to evaluate synchronization of neural activity in the same cortex (Gail et al. 2004) or between different regions either ipsilaterally or bilaterally (De Solages et al. 2010). Modulations of LFP coherence between pairs of cortical locations were seen in monkeys during visual perception test (Gail et al. 2004). Recently, increases in coherence of brain signaling among cortico-striatal-limbic circuits were seen in adaptation to multigenerational prenatal stress in adult rats (Skelin et al. 2015). These studies suggested a simple method to monitor the dynamic interplay between brain regions to confirm the increased communication between VTA and the NAc.

In conclusion, morphine administration was demonstrated to change LFPs in the NAc and locomotor activity in mice. Two distinct sub-gamma oscillations were separately viewed by using LFP analysis. Significant correlation between high gamma activity and distance travelled, phase-amplitude couplings and coherences were induced following morphine treatment. Therefore, these LFP patterns might represent the effects of morphine on the mesolimbic dopamine pathway and reward function.

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