

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

Chayaporn Reakkamnuan · Siriphun Hiranyachattada · Ekkasit Kumarnsit

© The Journal of Physiological Sciences 2015

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

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].

C. Reakkamnuan · S. Hiranyachattada · E. Kumarnsit (✉)
Department of Physiology, Faculty of Science, Prince of Songkla University (PSU), Hat Yai, Songkhla, 90112, Thailand
e-mail: kumarnsit.e@gmail.com

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

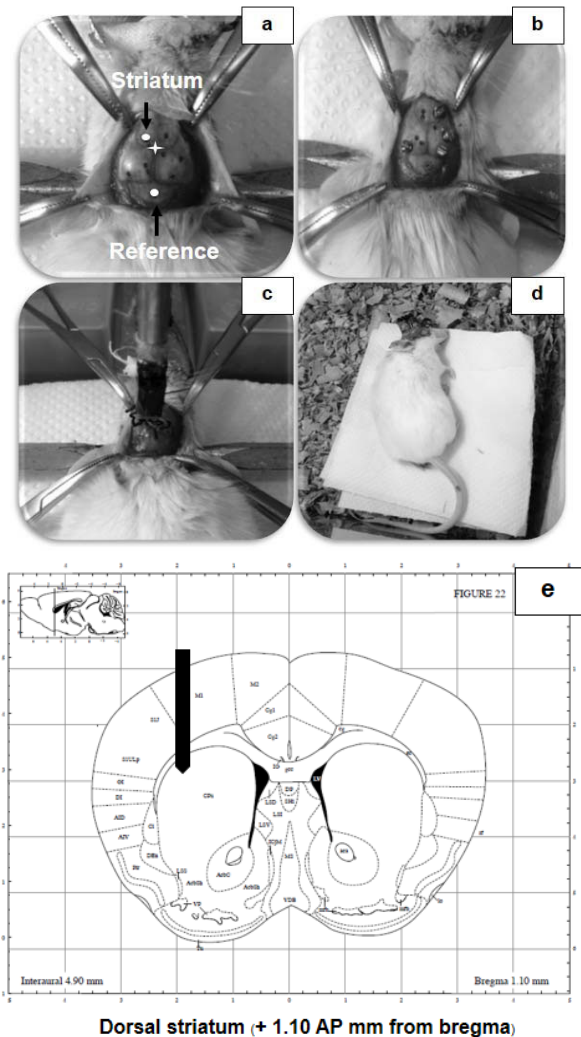


Fig. 1 Electrode implantation. (a) Striatum location of electrode implantation on the skull. Cross mark indicates bregma. (b) Stainless steel anchor screws were fixed on the skull. (c) Intracranial electrodes were implanted into the striatum and cerebellum (reference). (d) Dental acrylic was used to 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.

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.

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

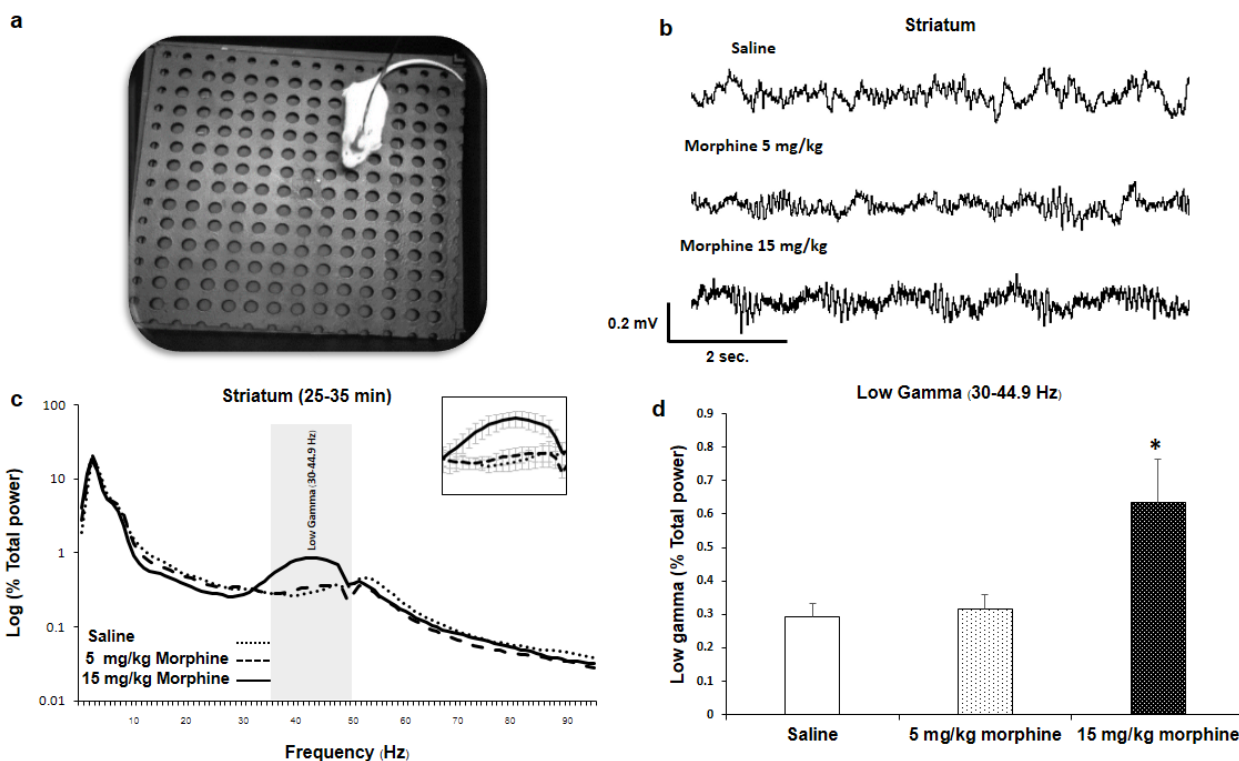


Fig. 2 Processes of LFP recording and analysis following acute morphine administration. (a) Individual animals were allowed to explore in the recording chamber. (b) Raw striatum LFP signals recorded from representative mice that received saline, 5 mg/kg morphine and 15 mg/kg morphine were displayed

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_{ai} -coupled μ -opiate

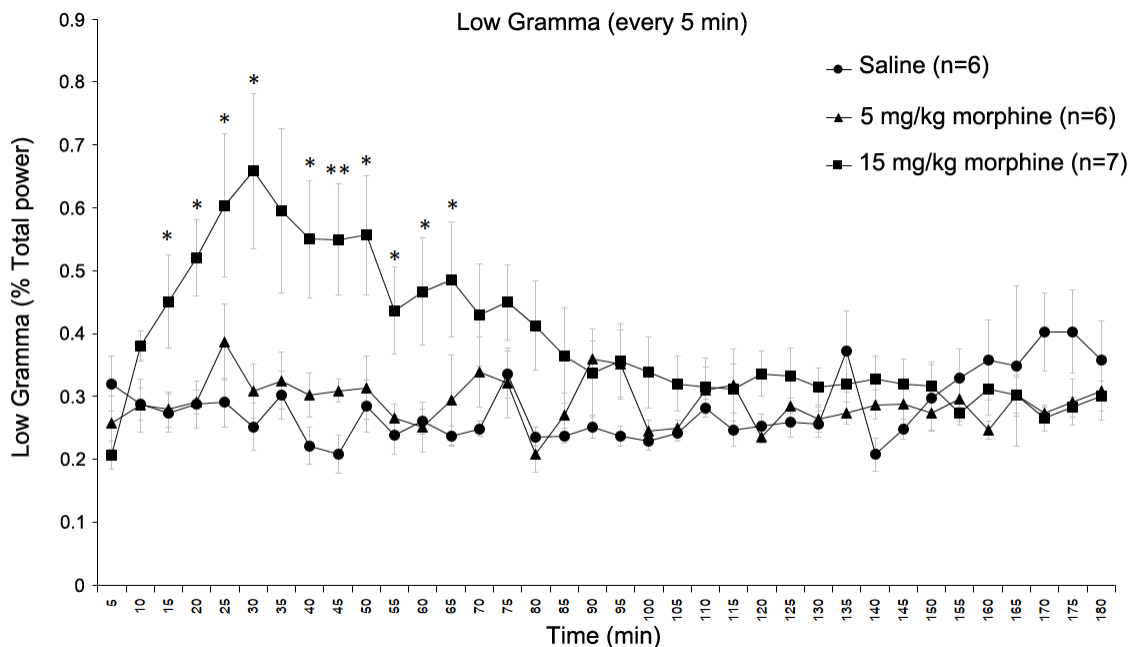


Fig. 3 The average percent total power of low gamma range were analyzed every 5 min period after injection of morphine (5 and 15 mg/kg) or saline. Data were compared with that of saline

control group using one-way ANOVA followed by Tukey's *post hoc* test. *, **: $P < 0.05$ and $P < 0.01$, respectively.

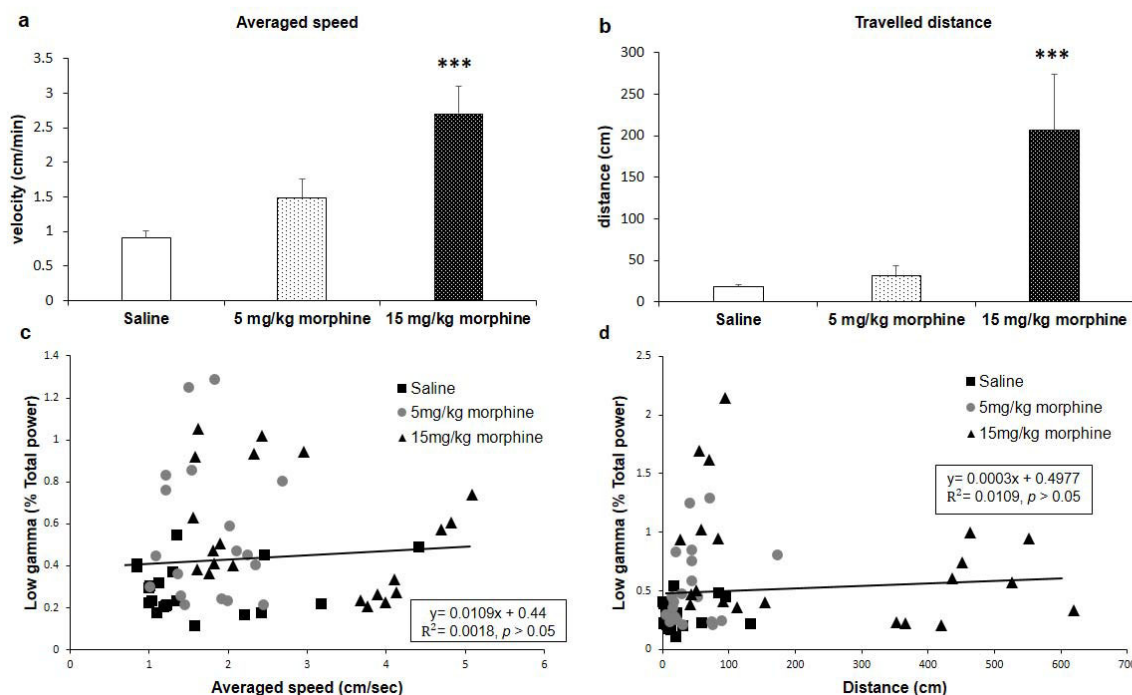


Fig. 4 Morphine induced averaged speed and travelled distance. (a) Averaged speed and (b) travelled distance during morphine administration expressed as mean ± S.E.M. (c) Regression analyses between striatum low gamma oscillation and

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.

Acknowledgements This work was supported by grants from the Graduated School and the Department of Physiology, Faculty of Science, Prince of Songkla University, Hatyai, Songkhla, Thailand.

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

References

- Volkow ND, Wang GJ, Fowler JS, Tomasi D, Telang F (2011) Addiction: Beyond dopamine reward circuitry. *PNAS* 108(37): 15037-15042.
- Olds J, Milner P (1954) Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. *J Comp Physiol Psychol* 47(6): 419-427.
- Spanagel R, Weiss F (1999) The dopamine hypothesis of reward: past and current status. *Trends Neurosci* 22(11): 521-527.
- Wise RA (2009) Roles for nigrostriatal — not just mesocorticolimbic — dopamine in reward and addiction. *Trends Neurosci* 32(10): 517-524.
- Wang H-L, Morales M (2008) The corticotropin releasing factor binding protein (CRF-BP) within the ventral tegmental area is expressed in a subset of dopaminergic neurons. *J Comp Neurol* 509(3): 302-318.
- Fallon JH, Loughlin SE (1995) Substantia nigra. In: Paxinos G (ed) *The Rat Nervous System*, 2nd edn. Academic Press, New York.
- Crow TJ (1972) A map of the rat mesencephalon for electrical self-stimulation. *Brain Res* 36(2): 265-273.
- Wise RA (1981) Intracranial self-stimulation: mapping against the lateral boundaries of the dopaminergic cells of the substantia nigra. *Brain Res* 213: 190-194.
- Delgado MR (2007) Reward-related responses in the human striatum. *Ann N Y Acad Sci* 1104: 70-88.
- Cheaha D, Bumrungsri S, Chatpun S, Kumarnsit E (2015) Characterization of *in utero* valproic acid mouse model of autism by local field potential in the hippocampus and the olfactory bulb. *Neurosci Res* 98: 28-34.
- Hnasko TS, Sotak BN, Palmiter RD (2005) Morphine reward in dopamine-deficient mice. *Nature* 438: 854-857.
- Gysling K, Wang RY (1983) Morphine-induced activation of A10 dopamine neurons in the rat. *Brain research* 277(1): 119-127.
- Chiara GD, Imperato A (1988) Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc Natl Acad Sci USA* 85(14): 5274-5278.
- Johnson SW, North R (1992) Opioids excite dopamine neurons by hyperpolarization of local interneurons. *J Neurosci* 12(2): 483-488.
- Albin RL, Young AB, Penney JB (1989) The functional anatomy of basal ganglia disorders. *Trends Neurosci* 12(10): 366-375.
- Lotharius J, Brundin P (2002) Pathogenesis of Parkinson's disease: dopamine, vesicles and α -synuclein. *Nat Rev Neurosci* 3: 932-942.
- Schultz W (1998) Predictive reward signal of dopamine neurons. *J Neurophysiol* 80(1): 1-27.
- Sun N, Li Y, Tian S, Lei Y, Zheng J, Yang J, Sui N, Xu L, Pei G, Wilson FAW, Ma Y, Lei H, Hu X (2006) Dynamic changes in orbitofrontal neuronal activity in rats during opiate administration and withdrawal. *Neuroscience* 138: 77-82.
- Kalenscher T, Lansink CS, Lankelma JV, Pennartz CMA (2010) Reward-associated gamma oscillation in ventral striatum are regionally differentiated and modulate local firing activity. *J neurophysiol* 103(3): 1658-1672.
- Masimore B, Schmitzer-Torbert NC, Kakalios J, Redish AD (2005) Transient gamma local field potential signal movement initiation in rats. *Neuroreport* 16(18): 2021-2024.
- Suto N, Wise RA, Vezina P (2001) Dorsal as well as ventral striatal lesions affect levels of intravenous cocaine and morphine self-administration in rats. *Neurosci Lett* 493(1-2): 29-32.
- Volkow ND, Wang GJ, Telang F, Fowler JS, Logan J, Childress AR, Jayne M, Ma Y, Wong C (2006) Cocaine cues and dopamine in dorsal striatum: Mechanism of craving in cocaine addiction. *J Neurosci* 26(24): 6583-6588.
- Herrmann CS, Munk MHJ, Engel AK (2004) Cognitive functions of gamma-band activity: memory match and utilization. *Trends in cognitive sciences* 8(8): 347-355.
- Masuda N (2009) Selective population rate coding: a possible computational role of gamma oscillations in selective attention. *Neural Comput* 21(12): 3335-3362.
- Tran AH, Tamura R, Uwana T, Kobayashi T, Katsuki M, Ono T (2005) Dopamine D1 receptors involved in locomotor activity and accumbens neural responses to prediction of reward associated with place. *PNAS* 102(6): 2117-2122.
- Tran AH, Tamura R, Uwana T, Kobayashi T, Katsuki M, Matsumoto G, Ono T (2002) Altered accumbens neural response to prediction of reward associated with place in dopamine D2 receptor knockout mice. *PNAS* 99(13): 8986-8991.