Differential local field potential oscillations in the dorsal striatum and locomotor activity induced by morphine and haloperidol in mice

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Dopamine (DA) depletion in the dorsal striatum underlies symptoms of basal ganglia pathologies, including Parkinson's disease (PD). Various drug compounds are used to enhance DA levels for therapeutic purposes. Understanding neural signaling and movement patterns associated with over- and under-stimulation of the DA system is essential. This study investigated striatal local field potential (LFP) oscillation and locomotor activity following treatments with morphine, a DA release enhancer, and haloperidol (HAL), a DA D2 receptor (D2R) antagonist in mice. After intracranial electrodes were placed into the dorsal striatum of male Swiss albino ICR mice, intraperitoneal injections of morphine or HAL were administered. LFP signals and spontaneous motor activity were recorded simultaneously. The results showed that morphine significantly increased locomotor speed, both low (30.3–44.9 Hz) and high (60.5–95.7 Hz) LFP gamma powers and delta (1–4 Hz)-gamma (30.3–95.7 Hz) phase-amplitude coupling. In contrast, HAL treatments were found to significantly decrease these parameters. Moreover, regression analyses also revealed significant positive correlations between locomotor speed and high gamma powers. Taken together, these results demonstrate opposite LFP oscillations in the dorsal striatum with low and high gamma activities, and delta-gamma couplings in response to a DA release enhancer and D2R antagonist by morphine and HAL, respectively. These parameters reflect fluctuation of neuronal activity in the dorsal striatum that might be useful for pathological research and drug discovery for PD.

Key words: dopamine, dorsal striatum, morphine, haloperidol, local field potential power, delta-gamma coupling

INTRODUCTION

Dopamine (DA) is a neurotransmitter with important roles in the organization of several brain functions, including motor control and reward-seeking behavior (Joshua et al., 2009; Schultz, 2010). The mesolimbic and nigrostriatal pathways are the two main DA systems. The mesolimbic pathway has dopaminergic neurons that originate in the ventral tegmental area (VTA) of the midbrain, and their projections reach the nucleus accumbens (NAc), cortex, amygdala, and hippocampus. Its major role is associated with reward function and motivation-driven behaviors (Björklund and Dunnett, 2007; Wise, 2009). The nigrostriatal system originates in the substantia nigra pars compacta (SNc) and projects to multiple regions, including the striatum's dorsolateral region, with a crucial role in cortico-striato-pallido-thalamo-cortica circuitry (Alexander et al., 1986). This DA pathway facilitates brain mechanisms for the behavioral selection of an optimal response and inhibits irrelevant ones (Mink,

2003). Notably, these 2 midbrain DA systems may overlap in functions (Wise, 2009). Dysfunction of DA transmission has profound consequences on the functions of downstream activities, including leading to the pathogenesis of several psychiatric and neurological illnesses such as Parkinson's disease (PD), depression, schizophrenia, and drug addiction, among others (Ko and Strafella, 2012). Changing DA release, metabolism, and receptor binding would have consequences in processing information in both normal and diseased conditions. The striatum is modulated by DA not only by the individual neurons but by the interactions between subpopulations of neurons as well (Humphries et al., 2009). Alterations of DA levels in the striatum cause changes to neuronal activity and eventually behavioral patterns. Several studies have shown that local field potential (LFP) reflects dopaminergic and non-dopaminergic activities within the midbrain (Humphries et al., 2009; Yael et al., 2013; Pasquereau et al., 2019).

The striatum is the principal input structure of the basal ganglia (BG) and has a large population of gamma-aminobutyric acid (GABA)-ergic projection neurons. Most of these striatal neurons are medium spiny neurons (MSNs) that project within BG networks (Czubayko and Plenz, 2002; Yael et al., 2013). Additionally, the striatum contains many small groups of interneurons, with two of the most studied types being GABAergic fast-spiking interneurons (FSIs) and cholinergic tonically active interneurons (Tepper et al., 2004). MSNs are separated into those that express D1 receptors (D1R) and scheme to the substantia nigra pars reticulata (SNr) and the interna globus pallidus part of direct output pathways, and MSNs that express D2 receptors (D2R) projecting to the externa globus pallidus part of indirect output pathways (Albin et al., 1989; Yael et al., 2013). However, the striatum receives afferents from the two main dopaminergic systems of the central nervous system (CNS) (Amalric and Koob, 1993). Reward-seeking behaviors and motor control are functionally mediated by the modulation of incoming DA transmission to the striatum (Cachope and Cheer, 2014). Glutamatergic inputs on striatal neurons are important factors that modify the output of the striatum (Britt et al., 2012; Paraskevopoulou et al., 2019). Moreover, the motor cortex gives strong excitatory projections that end at the striatum and provide it with information essential for controlling motor behavior (Gerfen, 1992). Additionally, striatal cholinergic neurons receive prominent inhibitory GABAergic inputs from direct and indirect pathway projection neurons (Gonzales et al., 2013). Previous studies have consistently suggested that GABAergic inputs strongly modulate the

striatum (Tepper et al., 2004; English et al., 2012) and that these afferents originate from intrinsic striatal sources, including GABAergic interneurons and axon collaterals of striatal output neurons (Tepper et al., 2004; Wilson, 2007).

LFP oscillations are changes in membrane potential synchronized across a population of neurons near a recording electrode. The oscillation reflects neural processes that underlie multiple functions, including cognition, emotion, and motor control (Nadjar et al., 2009; van der Meer and Redish, 2009; Herreras, 2016). An efficient way to identify brain mechanisms underlying behavioral traits is through the clinical profile of LFP oscillations (Cheaha et al., 2015; Nukitram et al., 2022) as, according to LFP studies, there is a link between features of LFP and movement deficits. Some aberrant LFP patterns were demonstrated to be indicators of PD (Nadjar et al., 2009) or Huntington's disease (Miller et al., 2011). These LFP oscillations returned to baseline patterns after the treatment with effective compounds for the pathologies. Therefore, the study of LFPs is a potentially valuable tool for clarifying the brain mechanisms in the diagnosis and investigation of neural diseases.

Opiates and antipsychotic drugs are known to intervene with dopaminergic neurons in the CNS that have different mechanisms (Iwatsubo and Clouet, 1975). Following morphine administration, extracellular DA concentration and DA metabolism were increased in the striatum via μ-opioid receptors (Kuschinsky and Hornykiewicz, 1974; Rougé-Pont et al., 2002). In brief, opioid receptors in the substantia nigra (SN) are mostly expressed in the GABAergic interneurons (Zhang et al., 2009). The activation of opioid receptors hyperpolarizes and inhibits GABAergic transmission and, therefore, would disinhibit SN dopaminergic neurons, increase DA neuronal firing and induce DA release (Johnson and North, 1992; Tan et al., 2010). In contrast, haloperidol (HAL), an antipsychotic, has a direct effect on the blockage of D2R. In rodents, systemic administration of HAL produces a depolarization blockade in nigral neurons, and a decreased firing rate, causing decreased extracellular levels of DA in the striatum (Drew et al., 1990; Johnson et al., 1992; Burkhardt et al., 2007).

This study aimed to determine whether LFP oscillatory patterns in the dorsal striatum are sensitive and unique in response to treatments with morphine and HAL for opposite changes in DA pathway activity. Morphine is a DA release enhancer, whereas HAL is a DA D2R antagonist. LFP signals from the dorsal striatum were recorded and analyzed to characterize neural signaling patterns in mice following the treatments.

METHODS

Animals

Adult male Swiss albino ICR mice (weighing 35-40 g) from Nomura Siam International Company, Bangkok, Thailand, were used in this study. At Prince of Songkla University's Southern Laboratory Animal Facility, all the animals were pre-handled for a week before the start of the experiment to reduce the effects of stress on the animals. Each animal was kept in individual stainless-steel cages (17 × 28 × 17 cm) with controlled temperature, humidity, and a 12/12 dark/light cycle (lights on at 7:00 a.m.). There was free access to commercial food pellets and filtered tap water. Experiments were performed between the hours of 8 a.m. and 4 p.m. between the periods of daylight. All procedures followed the European Science Foundation's guidelines and International Committee on Laboratory Animal Science, ICLAS (2004) guidelines (Van Zutphen, 2004). Various protocols for the care and use of experimental animals were approved and guided by the Animal Ethical Committee at Prince of Songkla University (project license number: 2562-01-072). All attempts were made to reduce the number of animals used and minimize animal suffering. Twenty-eight mice were used for the tests.

Drugs and chemicals

HAL (Sigma, St. Louis, MO, USA) and morphine sulfate (Zentiva, Hlohovec, Slovakia) were used. HAL was dissolved in 0.1 M hydrochloric acid (HCl) with heat and adjusted to pH 5.5-6. Morphine was dissolved in 0.9% w/v sodium chloride solution (NaCl). The drugs were prepared for intraperitoneal (i.p.) injection into animals.

Intracranial electrode implantation surgery

Electrodes were implanted into the animals' brains, followed by acclimatization to lessen stress during the behavioral test, and finally, the start of LFP recordings (Fig. 1). The method of electrode implantation has been previously described (Cheaha et al., 2015; Reakkamnuan et al., 2021; Nukitram et al., 2022). In brief, animals were administered a subcutaneous injection of atropine sulfate (0.01 mg/kg) to reduce secretions, then animals were anesthetized with an i.p. injection of zoletil (10 mg/kg) (Tiletamine-zolazepam, Vibac Ah, Inc., USA) and xylazine hydrochloride (3.4 mg/kg) (Xylavet, Sigma-Aldrich International

GmbH, Switzerland). After that, the animal's head was fastened with a stereotaxic frame through earpieces. The animal's scalp was shaved and cleaned before an anesthetic (20 mg/ml lidocaine) was injected into the skin around the midline of the scalp. A midline incision was made, and the scalp was pulled apart to expose the skull. Coordinates of the craniotomies were marked on the skull, and holes were drilled. A single stainless-steel electrode with a bare diameter of 0.008" (Coated-0.011") silver wire electrodes (A-M system, Sequim, WA, USA) were stereotaxically implanted to the left striatum (AP: +0.5 mm, ML: 2.0 mm, DV: 3.0 mm) utilizing bregma as the landmark according to the mouse brain atlas (Paxinos and Franklin, 2004). Electrodes were implanted in the midline of the skull, over the cerebellum, as a reference and ground electrode (AP: -6.5 mm, DV: 2 mm). To enhance the stability, additional holes were bored for stainless steel anchor screws. All electrodes were permanently attached to the cranium using dental acrylic (Unifast Trad, GC Dental Industrial Corp., Tokyo, Japan). Following the surgery, animals were housed individually in single cages for at least 2 weeks to recover fully from surgery. The antibiotic ampicillin (100 mg/kg) (General Drug House Co., Ltd., Bangkok, Thailand) and carprofen (10 mg/kg) (Best Equipment Center Co., Ltd., Thailand) were given subcutaneously once a day for three days to prevent infection and relieve pain. The precise location of the electrode tip placements was thoroughly checked after the experiment was complete with histological confirmation. After completion of the experimental session, the animals were deeply anesthetized with an i.p. overdose of zoletil and perfused transcardially with 0.9% saline, followed by 10% paraformaldehyde. The whole mouse brain was removed from the skull for fixation, embedded in paraffin, and the coronal brain sectioned through the striatum and verified in brain coronal slices 7 μm thick sections on a microtome. A mouse brain atlas was used to confirm the mark of the implanted electrode in the location of the striatum (Fig. 1).

The experimental setup and processing of LFP signals

Animals were habituated to the recording settings in a chamber for 210 min each day for three days before LFP recording in response to acute HAL or morphine administration. Then, animals were divided into 4 groups of 6-9 mice each and treated as follows. The control group received an i.p. injection of 0.1M HCl at pH 5.5-6 or 0.9% NaCl. The morphine group received morphine at 15 mg/kg (i.p.), and the two HAL groups
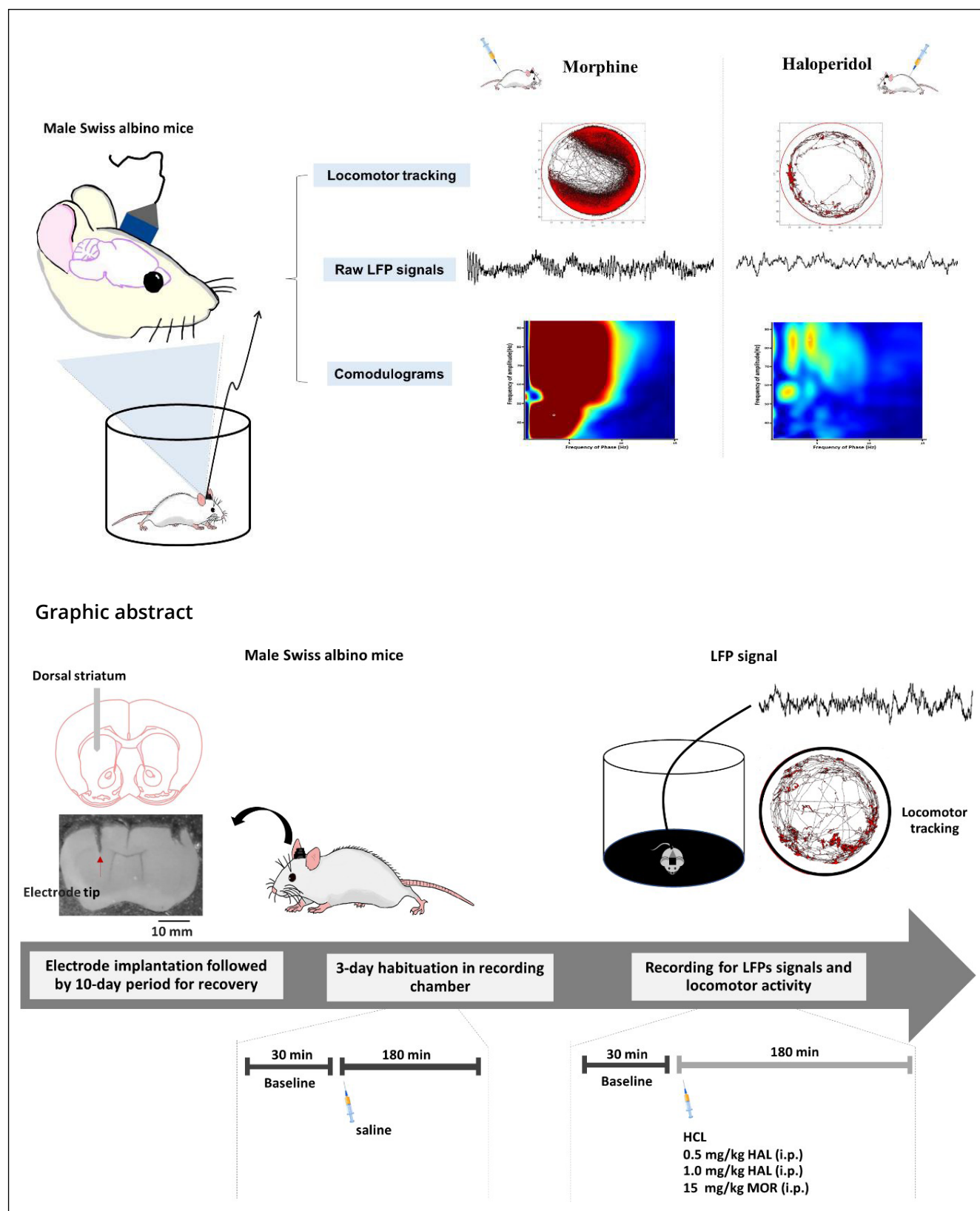
Fig. 1. Experimental protocol of behavioral and LFP testing for investigation of morphine and HAL. Animals were handled through the surgical processes for LFP electrode implantation into the dorsal striatum and a recovery period. After that, they were given habituation sessions to acclimatize to the recording system before the testing schedule for locomotor activity and LFP recordings. HAL: haloperidol; LFP: local field potential; MOR: morphine.

received HAL at 0.5 and 1.0 mg/kg (i.p.). Individual animals were placed in the recording chamber on the experimental day, where they were free to move and where both locomotor activity and LFP signals were recorded. Baseline activity was recorded for 30 min as the pre-drug period before i.p. injection of either 0.9% NaCl, morphine (15 mg/kg) or HAL (0.5 and 1.0 mg/kg). The post-drug recording was performed for 180 mins following the injection (Fig. 1).

Raw LFP signals from animals were amplified with a low pass of 1 kHz and a high pass of 0.3 Hz by Dual Bio Amp (AD Instruments, Castle Hill, NSW, Australia) for LFP signal processing. PowerLab 16/35 system (AD Instruments, Castle Hill, NSW, Australia) with 16-bit A/D transformed analog signals to digital data, and LabChart 7 pro software was used to save data on a computer. Signals were also gathered in sweeps with a period of 1.024 seconds and a sampling frequency of 2 kHz. To eliminate noise from power line artifacts, notch filtering at 50 Hz was used. The recorded files were visually considered, and only noise-free signals were used in the investigation. The data from 45-60 Hz were omitted from the study to avoid 50 Hz noise. The LFP signals were processed through a 1-100 Hz band-pass digital filter.

LFP signal analysis

Raw data were used to estimate the power spectral density (PSD) in LabChart, which was then transformed using the Hanning window cosine transform with a window overlap of 50 percent and a resolution of 0.9766 Hz. Using a fast Fourier transform (FFT) algorithm, power analysis frequencies were calculated for six discrete bands: delta (1.0-3.9 Hz), theta (4.7-8.5 Hz), alpha (9.8-12.7), beta (13.6-29.3 Hz), low gamma (gamma I) (30.0-44.9 Hz), and high gamma (gamma II) (60.5-95.7 Hz) (Nukitram et al., 2022). The data were normalized as percentages of baseline power for each frequency range. For the analysis of PSD, raw LFP signals from animals were selected from a full period of 180 min after treatment without taking into account differences in the amount of locomotor behavior. This was to screen changes in PSD of each frequency band after drug treatments every 30 min in the time domain. In addition, LFP signals were selected for epochs 1 min in the length of immobility every 30 min.

The phase-amplitude coupling (PAC) analysis was deployed for evaluating the interactions between oscillations of two different frequency ranges of LFP signal. A modulation index (MI) was computed and displayed in comodulograms or coupling maps to present fast wave amplitude modulated by a slow frequency phase.

Therefore, both amplitude and phase series were obtained first by filtering the LFP in the desired band frequency and then applying the Hilbert function to get its analytic representation. The instantaneous phase and amplitude series were extracted (Tort et al., 2010). All raw data analysis was performed *via* the toolbox Brainstorm3 software. The PAC in each group was separately calculated every 30 min for 180 min after drug treatment regardless of the movement or non-movement behavior of animals.

Recording and analysis of locomotor activity

A webcam set vertically on the top of the recording chamber was used to record the spontaneous movement of each animal. Videos that captured mouse reconnaissance were transferred to the computer through LabChart 7 Pro software. LFP oscillations and PAC patterns of mice receiving acute morphine and HAL administration were monitored using data selected every 30 minutes from those records. Each video recording of locomotor activities was evaluated to measure the animal movement for locomotor activity analysis. The process was tracked using the open-source toolbox OptiMouse, which captures the mouse body position (Ben-Shaul, 2017). Each frame of input data contained a black-and-white image (binary image) of the ground of the recording chamber and the body of a mouse, respectively. Therefore, the area of white pixels in the binary image was recognized as the body of an animal. Its boundary was indicated by the rectangle and the center of the rectangle was defined to represent the mouse's body point (Ben-Shaul, 2017). The alternations of locomotor speed in the assigned period in the recording chamber were illustrated and expressed as mean ± standard error of the mean (SEM).

Statistical analysis

The effects of morphine and HAL on striatal neuronal activity were averaged and expressed as the mean ± standard error of the mean (SEM) in percent baseline (before injection). ANOVA with one-way and multiple comparisons followed by Tukey's *post hoc* test was used to identify specific points of significance in the effects of morphine and HAL administration on LFP power, time domain, locomotor activity, and PAC. In addition, linear regression analyses were conducted between LFP power and locomotor activity, and between LFP power and delta-gamma PAC. At a *p*-value of <0.05, differences were considered statistically significant.

RESULTS

Effects of acute morphine and HAL on spontaneous locomotor activity

We first evaluated locomotor activity following treatments with morphine and HAL. Locomotor activities reflected sedative or hyperactive states of animals. The free movement patterns of mice during 3 h in an open field were analyzed. First, locomotor activity patterns were compared among groups using locomotor tracking images (Fig. 2A). Locomotor levels appeared to be increased by 15 mg/kg morphine and decreased by both 0.5 and 1.0 mg/kg HAL compared to the control level. Therefore, data were analyzed in terms of locomotor speed (cm/min) every 30 minutes (Fig. 2B). One-way ANOVA revealed significant differences during the periods of 30-60 min ($F_{(3,27)}$=59.628; P<0.001), 60-90 min ($F_{(3,27)}$=64.452; P<0.001), 90-120 min ($F_{(3,27)}$=49.951; P<0.001) and 120-150 min ($F_{(3,27)}$=23.906; P<0.001). Multiple comparisons indicated significant increases induced by morphine during these periods. Conversely, both 0.5 and 1.0 mg/kg HAL treatment were found to significantly decrease locomotor speed during the periods of 60-90 min ($F_{(3,27)}$=64.452; P<0.001) and 90-120 min ($F_{(3,27)}$=49.951; P<0.05). One-way ANOVA further revealed a significant difference when analyzed for a total of 180 min ($F_{(3,27)}$=36.834) (Fig. 2C). Moreover, the average speed of animals was significantly increased by morphine and decreased by both 0.5 and 1.0 mg/kg HAL treatments.

Effects of acute morphine and HAL on striatal LFP oscillations and power spectrums

Raw LFP signals following treatments with morphine and HAL were visualized in tracings (Fig. 3A) and spectrograms. The color bar of the spectrogram encodes LFP frequency power levels; the colors at the top of the color bar indicate frequency content with higher powers, whereas colors at the bottom indicate frequency content with low powers (Fig. 3B). Morphine seemed to induce the fast wave (~30.3-65.0 Hz) activity seen in these images, whereas HAL treatments did not produce an obvious change that could be distinguished by visualization. Therefore, data were expressed as LFP power spectrums in the frequency domain (Fig. 3C) and analyzed for the frequency ranges of theta, alpha, beta, and low and high gamma (Fig. 3D-H). One-way ANOVA revealed significant differences for alpha ($F_{(3,27)}$=6.129), beta ($F_{(3,27)}$=6.016), low ($F_{(3,27)}$=25.481) and high ($F_{(3,27)}$=13.340) gamma ranges. Multiple comparisons confirmed a significant decrease produced by 15 mg/kg morphine in alpha and beta waves, while neither 0.5 nor 1.0 mg/kg HAL produced significant differences. In addition, multiple comparisons indicated a significant decrease produced by 1.0 mg/kg HAL and an increase by 15 mg/kg morphine in the low gamma wave and high gamma power was significantly increased by 15 mg/kg morphine.

Therefore, alpha, beta, low and high gamma powers were quantitated separately and analyzed every 30 min in the time domain (Fig. 4A-B and 5A-B). One-way ANOVA revealed significant differences during the periods of 0-30 min ($F_{(3,27)}$=10.799), 30-60 min ($F_{(3,27)}$=20.184), 60-90 min ($F_{(3,27)}$=8.821), 90-120 min ($F_{(3,27)}$=6.266), 120-150 min ($F_{(3,27)}$=12.455) and 150-180 min ($F_{(3,27)}$=3.299) for alpha power. Multiple comparisons indicated significant decreases induced by 15.0 mg/kg morphine from 0-180 min. However, both doses of HAL did not produce significant differences. Similar results were observed for beta power, with multiple comparisons indicating significant decreases induced by 15.0 mg/kg morphine from 0-180 min, with no change from the control group for 0.5 and 1.0 mg/kg of HAL. Moreover, one-way ANOVA revealed significant differences during the periods of 0-30 min ($F_{(3,27)}$=34.021), 30-60 min ($F_{(3,27)}$=181.962), 60-90 min ($F_{(3,27)}$=52.834), 90-120 min ($F_{(3,27)}$=14.157) and 120-150 min ($F_{(3,27)}$=4.789) for low gamma power. Multiple comparisons indicated significant increases induced by 15.0 mg/kg morphine from 0-90 min and a decrease induced by 0.5 mg/kg HAL from 30-120 min and by 1.0 mg/kg HAL from 0-150 min. For high gamma power, significant differences were found during periods of 60-90 min ($F_{(3,27)}$=8.135), 90-120 min ($F_{(3,27)}$=24.879) and 120-150 min ($F_{(3,27)}$=20.539). Multiple comparisons confirmed significant increases induced by 15 mg/kg morphine from 90-150 min. High gamma power was significantly decreased by 0.5 mg/kg HAL from 90-150 min and by 1.0 mg/kg HAL from 60-150 min. Furthermore, it was noted that low and high gamma activities responded differentially to the treatments. Low gamma power was significantly induced as quickly as in the first 30 mins, whereas high gamma needed a longer time to be affected by the treatments. The small insets show the effects of morphine and HAL on low and high gamma powers during immobile periods when animals did not move. Significant changes in low gamma powers remained, whereas that of high gamma powers was absent.

Correlations between locomotor velocity and striatal gamma (low and high) powers following treatment with morphine and HAL

This study demonstrated the obvious opposite effects of morphine and HAL on low and high gamma

and demonstrated changes to low and high gamma at different times. Therefore, gamma oscillations in this brain region are controlled by more than one mechanism. Thus, we evaluated low and high gamma frequency correlation with locomotor velocity following treatments with morphine and HAL. The correlations
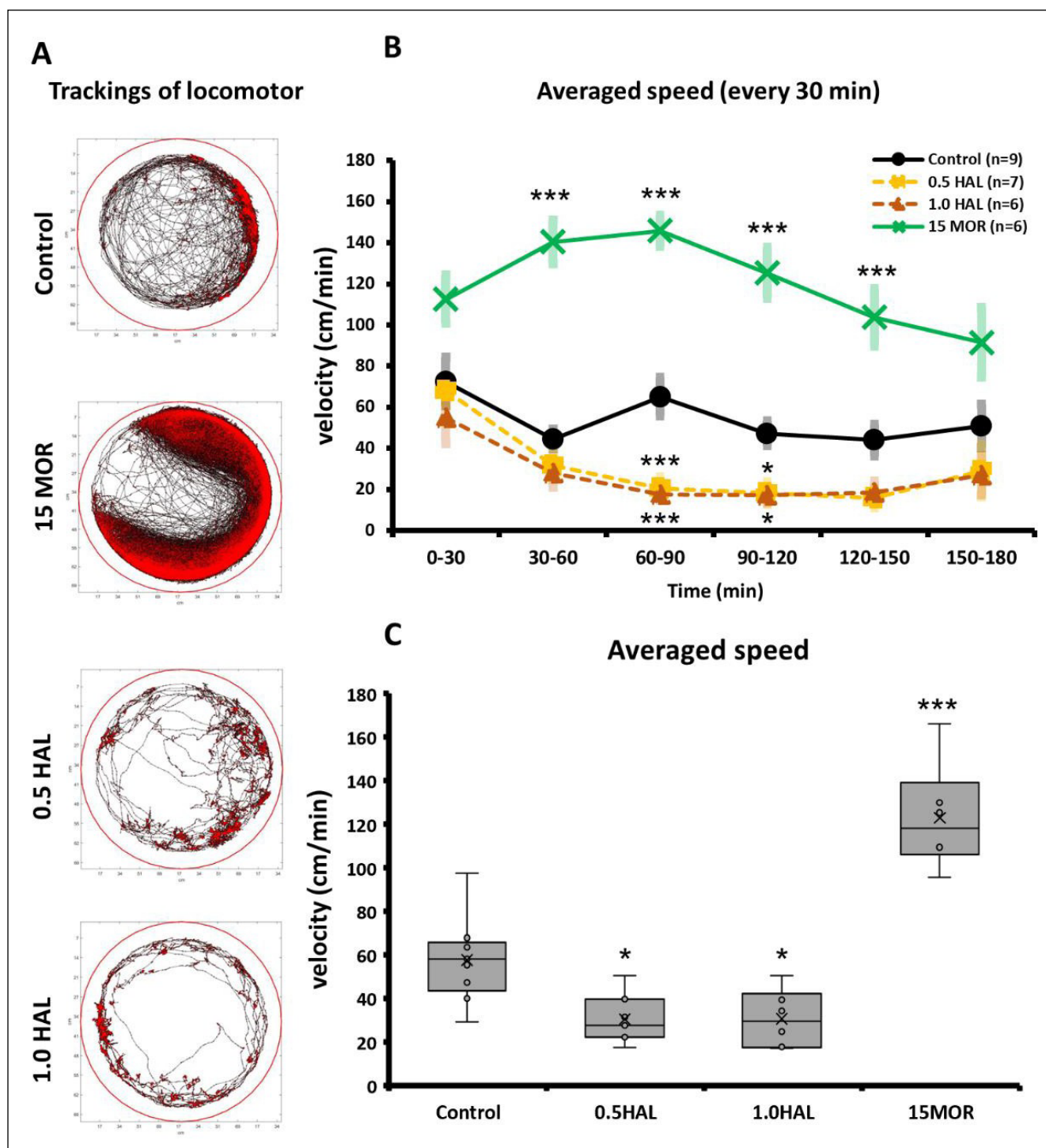

Fig. 2. Locomotor activity of mice in a recording chamber following treatments with morphine and HAL. (A) Locomotor tracking of representative animals from each group was shown in comparisons among groups. (B) Locomotor speed in each group was calculated during every 30-min block. (C) Mean ± SEM values of averaged speed during 180-min of exploratory behavior in a recording chamber were analyzed. Data are expressed as mean ± SEM and compared with that of the control group using one-way ANOVA followed by Tukey's *post hoc* test. *, ***$P \leq 0.05$, 0.001.
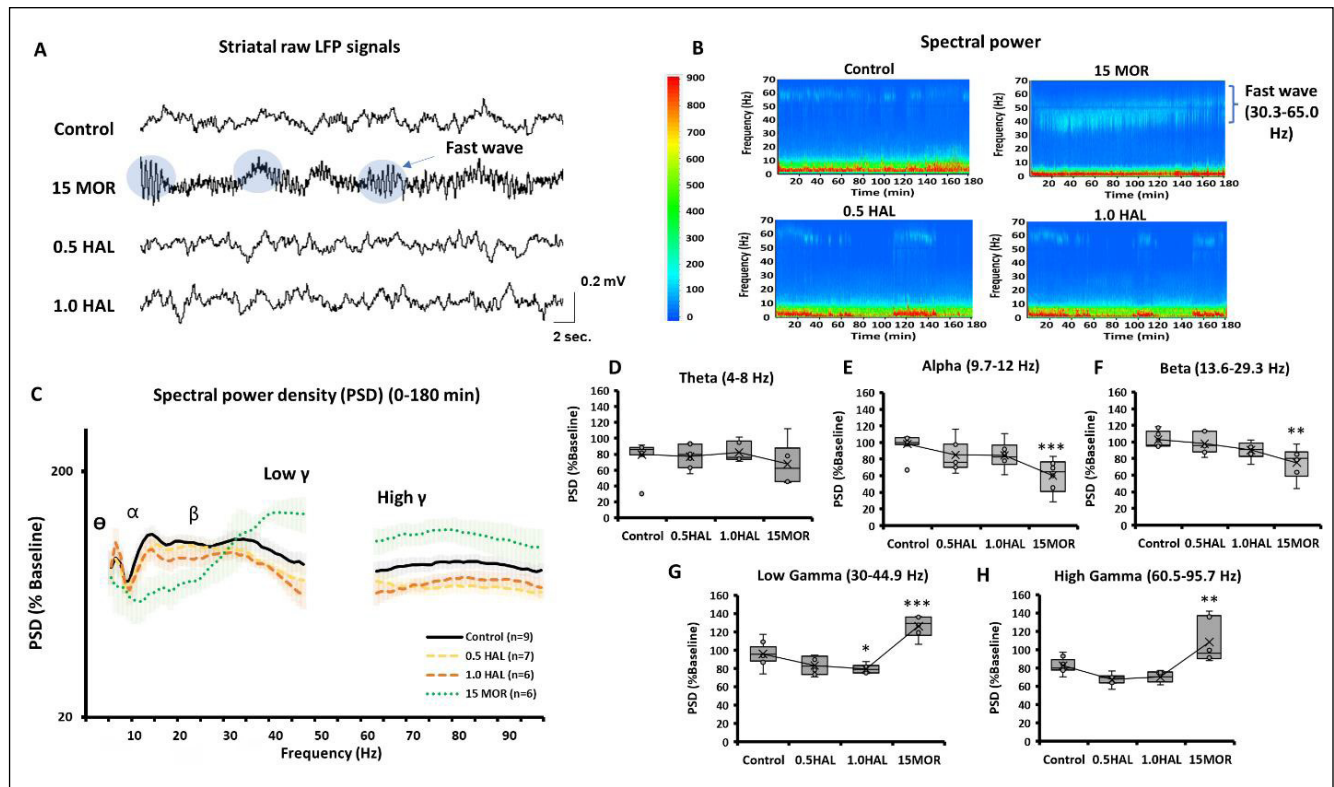
Fig. 3. Effects of the treatments with morphine and haloperidol (HAL) on LFP signals in the dorsal striatum. Raw LFP signals recorded from representative mice that received 0.1M HCL, 15 mg/kg morphine, and 0.5 and 1.0 mg/kg HAL were displayed (A). Spectrograms of striatal LFPs were expressed in time-domain (B) and frequency-domain (C) during a 180-min period following the treatments. Averaged percent baseline of theta (D), alpha (E), beta (F), low (G) and high (H) gamma ranges were expressed as mean ± S.E.M. *, **, ***$P \leq 0.05$, 0.01, 0.001, respectively, compared with control levels (one-way ANOVA followed by Tukey's *post hoc* test).

between averaged speed and gamma (low and high) powers during 60-90 min and 120-150 min periods were separately analyzed, finding no significant correlations were seen for both low and high gamma powers during 60-90 min (Fig. 6A and B). During 120-150 min, no significant correlations were seen for low gamma, although high gamma powers of all groups had significant correlations with average speed (control: R^2=0.2051, p<0.05; 0.5 HAL: R^2=0.4916, p<0.01; 1.0 HAL: R^2=0.3198, p<0.05; 15 MOR: R^2=0.3670, p<0.05) (Fig. 6C, D, respectively).

Phase-amplitude coupling (PAC) in the striatum following treatment with morphine and HAL

Low-frequency modulation of gamma activity was analyzed every 30 min and comodulograms for the periods 30-60 min and 60-90 min from representative animals were compared between groups (Fig. 7A). The couplings between a wider range of low frequency for phase and a wider range of frequency for ampli-

tude appeared during 30-60 and 60-90 min following 15.0 mg/kg morphine treatment compared to control levels. The couplings were decreased following both 0.5 and 1.0 mg/kg HAL treatments. Therefore, data were quantitatively analyzed as the % baseline modulation index of delta-gamma PAC for 30-60 min and 60-90 min (Fig. 7B, C). One-way ANOVA revealed significant differences during 30-60 min ($F_{(3,27)}$=38.152) and 60-90 min ($F_{(3,27)}$=35.937) periods. Furthermore, multiple comparisons indicated increases produced by 15.0 mg/kg morphine and decreases produced by both 0.5 and 1.0 mg/kg HAL during these periods. The data of delta-gamma PAC analyzed every 30 min were also plotted (Fig. 7D), finding significant differences only during the periods of 30-60 min ($F_{(3,27)}$=23.691) and 60-90 min ($F_{(3,27)}$=25.379). Regression analyses between delta-gamma PAC and gamma (low and high) during 60-90 min were performed and plotted (Fig. 8A, B). Delta-gamma PAC was significantly correlated with low gamma (control: R^2 =0.4049, p<0.05; 0.5 HAL: R^2=0.3063, p<0.05; 1.0 HAL: R^2=0.0393, p>0.05; 15 MOR: R^2=0.3644, p<0.05) but not with high gamma.
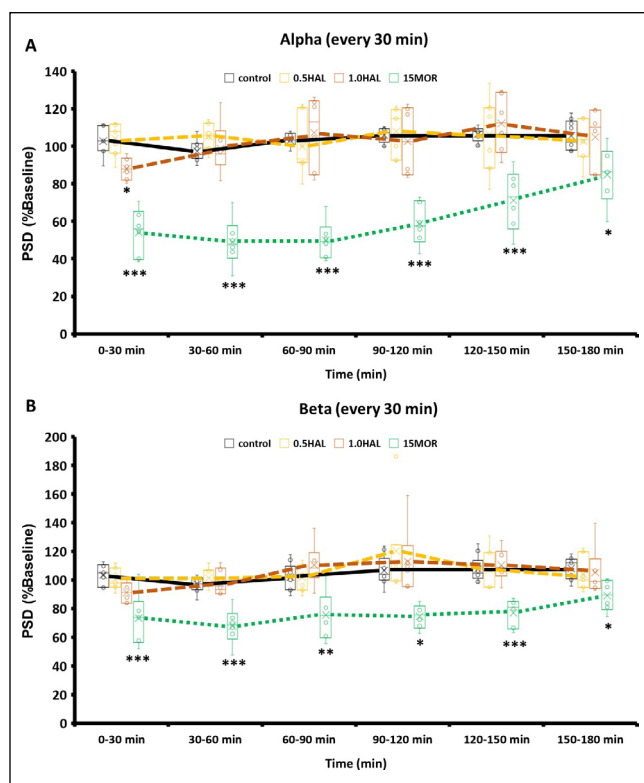
Fig. 4. Averaged percent baseline power every 30-min bin of alpha (A) and beta (B) frequency ranges following treatment with 15 mg/kg morphine and 0.5 and 1.0 mg/kg HAL in freely moving mice. Data were compared with control levels using one-way ANOVA followed by Tukey's *post hoc* test. *, **, ***: $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively.
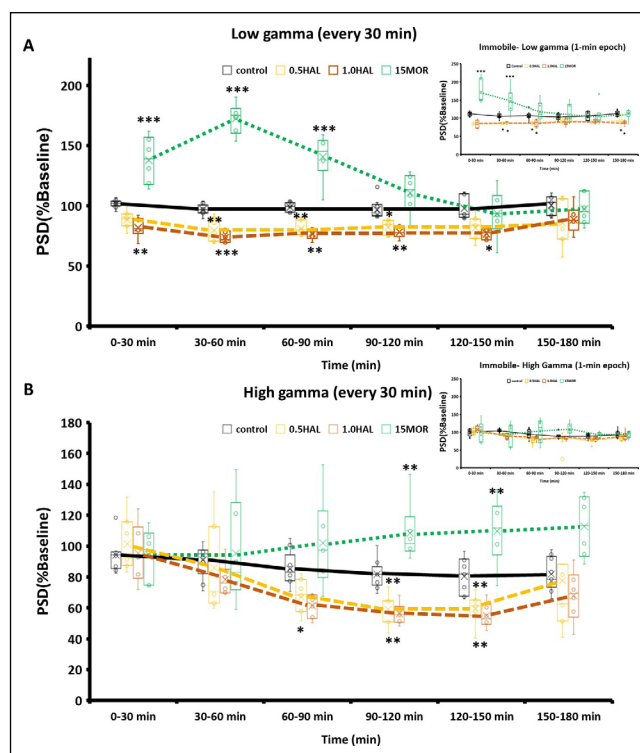

Fig. 5. Averaged percent baseline power every 30-min bin and 1-min epoch during immobility of low gamma (A) and high gamma (B) frequency ranges following treatment with 15 mg/kg morphine and 0.5 and 1.0 mg/kg HAL in freely moving mice. Data were compared with control levels using one-way ANOVA followed by Tukey's *post hoc* test. *, **, ***: $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively.

DISCUSSION

The present study investigated the effects of acute treatments with morphine and HAL on LFP oscillation in the dorsal striatum and correlations between locomotor activity and LFP spectral powers. Morphine increases DA release in the dorsal striatum, whereas HAL blocks DA D2R. The present findings demonstrated the opposite effects of these 2 substances on all parameters evaluated. Morphine enhanced both low and high gamma powers that peaked at approximately 30-60 min and 120-150 min respectively. Conversely, the blockade of DA D2R resulted in decreases in both low and high gamma activities. Moreover, delta-gamma PAC also exhibited opposite effects in responses to the DA release enhancer and DA D2R blockade. Additionally, regression analyses demonstrated positive correlations between locomotor speed and high gamma oscillations during 120-150 min.

Previously, systemic administration of opioids increased the synthesis, metabolism and release of DA in the striatum (Di Chiara and Imperato, 1988). The ac-

tivation of DA cells *via* opioid-class receptors in mice pre-synaptically inhibits GABAergic transmission that negatively regulates DA cell firing (Johnson and North, 1992; Tan et al., 2010). This, in turn, resulted in an increased firing rate of DA neurons in the VTA (Chen et al., 2015) and SNc (Gysling and Wang, 1983). However, large systemic doses or direct administration of morphine into the striatum decreased the firing rate, decreased striatal DA release, and induced behavioral suppression and catalepsy in a similar manner to HAL (Moleman and Bruinvels, 1979; Piepponen et al., 1999; Fischer et al., 2002). In contrast, low doses of systemic morphine injections enhanced striatal DA release and increased locomotor activity (Ostrowski et al., 1982; Piepponen et al., 1999). These effects were associated with an affinity to opioid receptors on striatal neurons (Di Chiara and Imperato, 1988). In rodents, systemic administration of HAL decreased firing rate and DA release in the striatum that results in akinesia and rigidity of movements due to the blockage of D2 DA receptors in the nigrostriatal pathway (Burkhardt et al., 2009). Thus, low doses of morphine (15 mg/kg) and 0.5
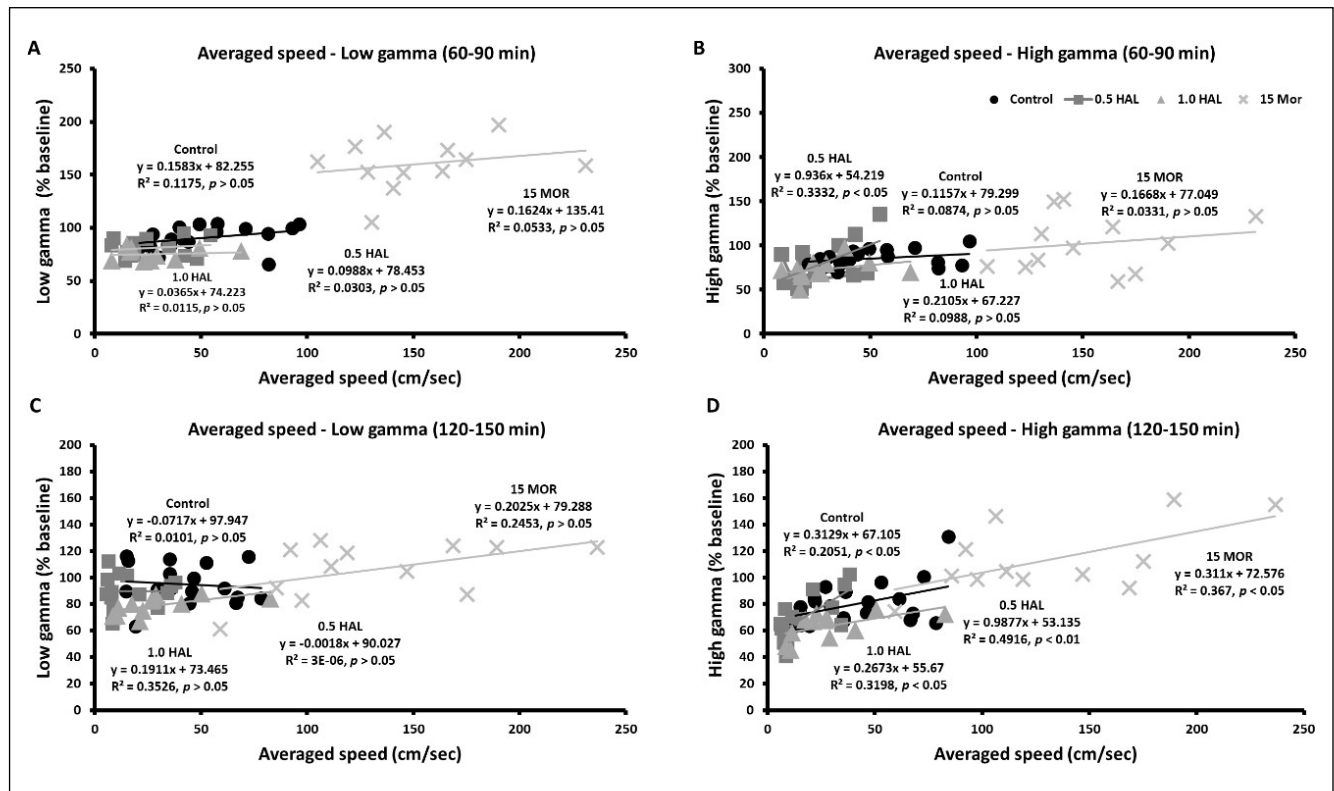
Fig. 6. Regression analyses between gamma (low and high) powers in the dorsal striatum and locomotor speed during 60-90 min (A and B) and 120-150 min (C and D) following treatment with 15 mg/kg morphine and 0.5 and 1.0 mg/kg HAL.

and 1.0 mg/kg HAL appeared to properly stimulate and suppress neuronal activity, respectively, in the dorsal striatum as seen in the resulting LFP patterns.

Furthermore, we demonstrated the opposite effects of morphine and HAL on locomotor activity, results that are consistent with earlier research (Park and Yoo, 2003; Reakkamnuan et al., 2017). The μ-opioid receptor is thought to play an essential role in locomotor activity produced by morphine (Park and Yoo, 2003). Reduced locomotor activity was observed due to either DA D1R (Tran et al., 2005) or D2R (Tran et al., 2002) knockout (D1RKO or D2R-KO, respectively) in the nigrostriatal system or blocking of either DA D1R or D2R (Burkhardt et al., 2009; Kharkwal et al., 2016). Previously, although DA-depleted mice were unable to elicit a normal locomotor response, they were shown to significantly increase locomotor activity following morphine administration (Hnasko et al., 2005). This study suggested that morphine as a DA release enhancer and HAL as a DA D2R antagonist are likely to stimulate or inhibit locomotor activity, respectively, through DA neurotransmission.

Morphine and HAL affected LFP oscillations in the dorsal striatum. Low and high gamma activities were sensitive to the administrations in mice, with the results showing opposite effects on low and high gamma

oscillations. Furthermore, the low gamma oscillation responded before high gamma to the administrations, highlighting that gamma oscillations in this part of the brain are controlled by more than one mechanism. Based on the experimental evidence, gamma oscillations are found in several brain regions and are related to a variety of neuronal processes that occur within neuronal networks (Buzśaki and Wang, 2012). Treatment with morphine was found to increase low gamma power in the dorsal striatum (Reakkamnuan et al., 2015) and increase low and high gamma in the ventral striatum of mice (Reakkamnuan et al., 2017). Previously, FSIs were found to generate gamma-band fluctuations through an intrinsic membrane mechanism (Bracci et al., 2003). This mechanism of gamma band oscillation is mediated *via* GABA$_A$ receptors (Penttonen et al., 1998; Hasenstaub et al., 2005; Berke, 2011; Buzśaki and Wang, 2012) in combination with a fast-activating, slow-inactivating potassium current (the D-type current) to allow FSIs to produce gamma oscillations (Chartove et al., 2020). In brief, FSIs form functional electrical connections with each other. The correlation between striatal rhythms and DA modulation in striatal microcircuits has also been demonstrated previously (Chartove et al., 2020).
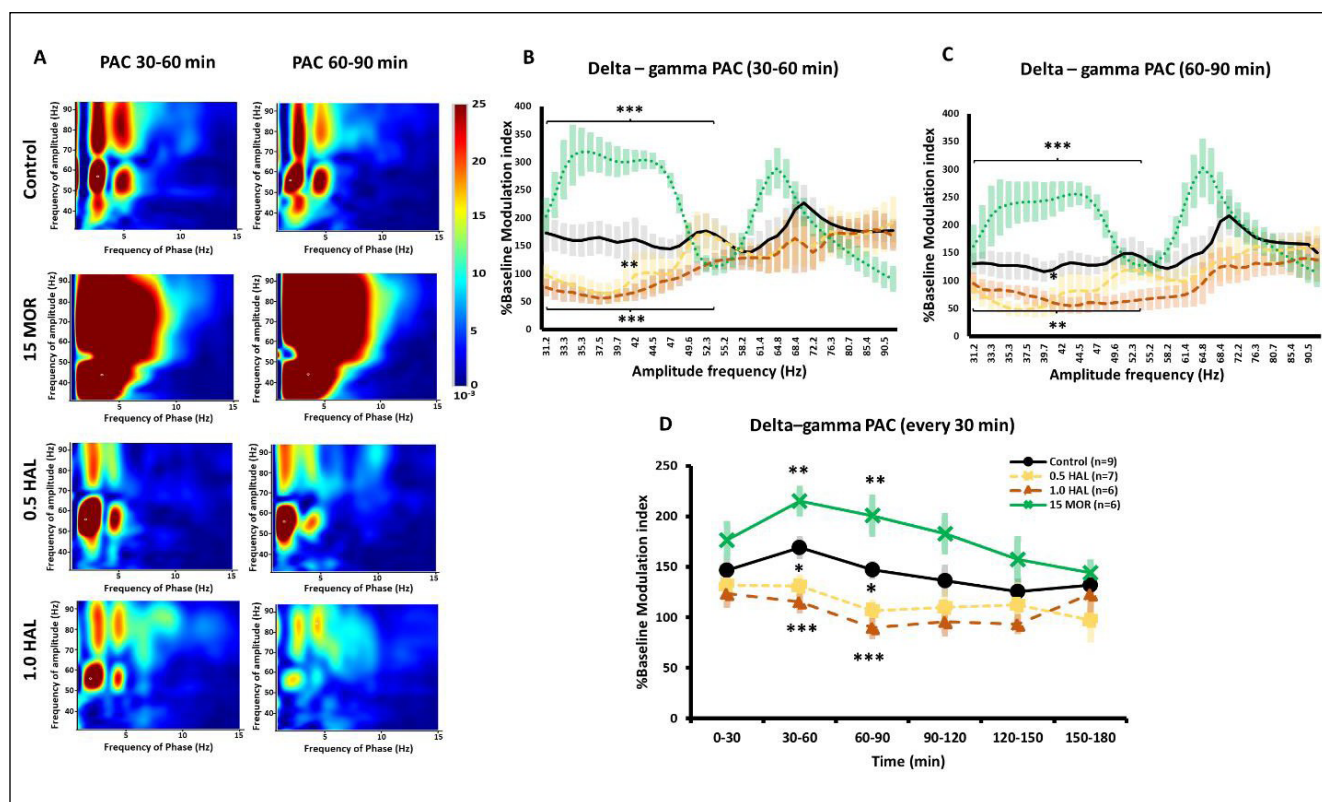
Fig. 7. Phase-amplitude couplings (PAC) of the dorsal striatum LFPs following treatments with 15 mg/kg morphine, 0.5 and 1.0 mg/kg HAL. The comodulograms show cross-frequency couplings in the frequency domain. The grey scales indicate the degrees of coupling or modulation index (MI) between phase-modulating slow frequency (1-15 Hz) and amplitude-modulated gamma frequency (30-100 Hz) (A). Delta phase (1-4 Hz) was selected for modulation of gamma amplitude during 30-60 min (B) and 60-90 min (C). Mean ± S.E.M. values of MI of delta-gamma PAC every 30-min following the treatments (D). Data were compared with that of the control group using one-way ANOVA followed by Tukey's *post hoc* test. *, **, ***: $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively.
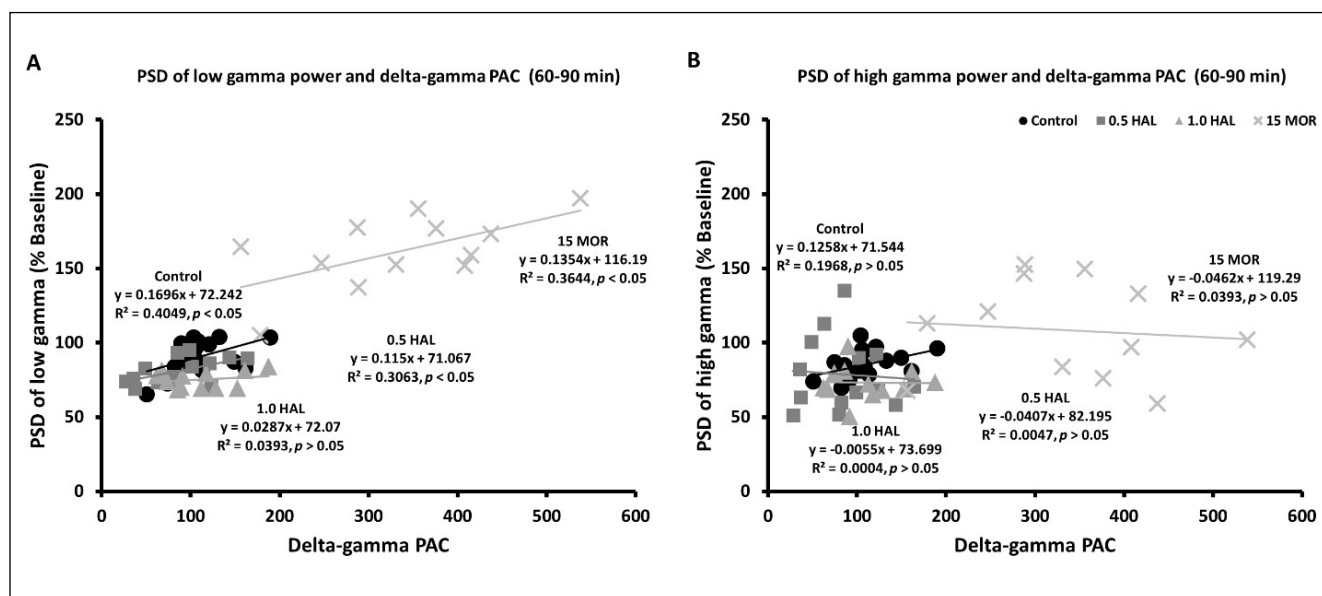

Fig. 8. Regression analyses between delta-gamma PAC and gamma (low and high) powers in the dorsal striatum (A and B) during 60-90 min following treatment with 15 mg/kg morphine and 0.5 and 1.0 mg/kg HAL.

Interestingly, regression analyses demonstrated that high gamma oscillations in the dorsal striatum were significantly correlated with locomotor speed following morphine and HAL treatments. During the immobile phase following morphine and HAL treatments, the changes in low gamma power remained, whereas the decreases in high gamma were abolished. Ultimately, the activity of the dorsal striatum is associated with motor executive functions, including action selection, decision-making, and movement initiation (Groenewegen, 2003). This suggests that multiple gamma oscillations are linked with various motor control processes. Previously, changes in firing rate in FSIs differentially contributed to low and high gamma powers (van der Meer and Redish, 2009), and theoretically, individual FSIs can exhibit a wide range of oscillation frequencies and can switch between them. Low and high gamma may be distinguished by differences in afferent inputs, neuromodulation, intrinsic properties, or their interaction with each other (van der Meer and Redish, 2009). In correlation with locomotor speed, gamma rhythms in different frequency ranges indicate different behavioral states. Previously, low gamma power in the dorsal striatum was highest during the T-maze task performed at the reward site and movement initiation (Tort et al., 2008; Cunningham et al., 2021), whereas high gamma power was increased throughout the maze runs (Tort et al., 2008). In a rat model of PD with unilateral DA depletion in the sensorimotor striatum, the oscillation in the low gamma range was selectively amplified, whereas high gamma activity was weakened during task performance (Lemaire et al., 2012). These findings support the link between locomotor speed and gamma frequency in the dorsal striatum in response to DA modulation. In addition, the present study demonstrated decreased alpha and beta activities in mice following morphine administration. In some studies, a wide range of beta waves is 8-30 Hz, with its low end occasionally recognized as an alpha wave. In short, beta oscillations have been linked to attention, motor set, preparation, or expectation in sensorimotor circuits (Courtemanche et al., 2003). In healthy individuals, oscillatory activity in the beta range was involved in the "idling" state of motor circuits (Pfurtscheller et al., 1996). However, in the Parkinsonian state, the basal ganglia showed very intense beta band (and alpha band) oscillations (Raz et al., 2001; Brazhnik et al., 2021). Thus, it seems plausible that enhanced synchronization of beta activity in the basal ganglia would hinder or delay movement initiation in PD patients leading to bradykinesia and rigidity (Hutchison et al., 2004). Moreover, in both healthy and disease conditions, striatal beta power had a well-established negative association with DA and locomotion, whereas striatal gamma power had a positive link with

both (Jenkinson and Brown, 2011). Moreover, with the simulations of high DAergic tone, FSI-generated high gamma frequencies periodically inhibited MSN-generated beta oscillations (Chartove et al., 2020). Therefore, morphine may enhance locomotor activity through decreased beta activity and increased gamma activity.

Multiple LFP oscillatory activities allocated to distinct frequency bands are not completely independent. One type of interaction is modulated by low-frequency rhythms that moderate the amplitude of higher-frequency oscillations. Moreover, cross-frequency interactions of neural activity within brain networks were found to have functional significance in cognitive performances, including active navigation and decision-making in T-maze tasks (Tort et al., 2008). The present study demonstrated that changes in the delta phase modulated the amplitude of gamma oscillations in the dorsal striatum following morphine and HAL treatments. Delta-gamma PAC was significantly increased during 30-90 min by 15 mg/kg morphine. In the same period, delta-gamma PAC decreased by 0.5 and 1.0 mg/kg HAL, and changes to PAC in the dorsal striatum were simultaneous with that of low gamma frequency. During the 60-90 min period (likely to have a peak of low gamma within), the induced delta-gamma PAC was significantly correlated with low gamma powers. These neural activities were confirmed to be related to DA neurotransmission in the dorsal striatum. Interestingly, intracranial electroencephalogram (iEEG) recordings of patients showed that a shift in the delta-gamma PAC marker was consistent across epileptic seizures in patients (Grigorovsky et al., 2020), with seizures causing major alterations in various activities of the dopaminergic system (such as DA release, metabolism, and receptor binding) in both humans and experimental animals (Rezaei et al., 2017). However, the optogenetic study showed that DA release in the medial prefrontal cortex (PFC) shifts phase-amplitude comodulation from theta-gamma to delta-gamma (Andino-Pavlovsky et al., 2017). Overall, changes in delta-gamma PAC in the dorsal striatum and its correlation with low gamma frequency are sensitive to treatments with exogenous substances that affect DA release.

CONCLUSIONS

Altogether, the present findings demonstrated clear effects of DA release enhancer and DA D2R blockade on LFP power spectrums and delta-gamma PAC in the dorsal striatum and locomotor activity in mice. This study highlighted the opposite responses induced by morphine and HAL seen in the unique patterns of LFP oscillations and correlations between LFP activities and lo-

comotor speed. The data in response to DA release and blockade might be used as indicators of dopaminergic activity in the nigrostriatal pathway. These findings may also be useful in testing the level of dopaminergic activity in the striatum for diagnosis and the progress of dopaminergic dysfunction.

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