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A comparison between spontaneous electroencephalographic activities induced by morphine and morphine-related environment in rats

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ABSTRACT

Previous studies demonstrated that drug cues could elicit drug-like or withdrawal-like effect, both subjectively and physiologically. However, few studies have compared the central activities induced by a drug-related environment and the drug itself. The aim of this study was to observe and compare electroencephalographic (EEG) changes induced by acute morphine administration and by the morphine-related environment. EEG activities were recorded via twelve skull electrodes scattered on the left and right cortex in conscious, freely moving rats, either after acute morphine administration or after successful training of conditioned place preference. Acute administration of morphine (0.1, 0.5, 1, 5, 10, 20 mg/kg, i.p.) produced an increase in absolute EEG power in the delta, theta, alpha1, alpha2, beta1, and beta2 bands, as well as a decrease in the gamma band. Topographic mapping revealed a maximal increase in the lateral leads in the theta band and a maximal change in the centro-frontal region in the remaining bands. After place conditioning training, the morphine-related environment induced a diffuse decrease in absolute power in the delta, theta, alpha1, alpha2, beta1, and beta2 bands, which was opposite to the changes induced by acute morphine administration. In addition, the changes in relative power induced by the two situations also diverged. These results indicate that the central mechanisms underlying the motivation of morphine-induced place preference may be somehow different from those underlying the reward effects produced by acute morphine administration.

1. Introduction

For drug abusers, drug-related cues are perceived as 'attractive'. Many research studies have demonstrated attentional, evaluative, and approach biases for drug-related stimuli in abusers of alcohol (Cox et al., 2003; Duka and Townshend, 2004; Lusher et al., 2004; Field et al., 2005a), nicotine (Waters et al., 2003; Field et al., 2005b), cocaine (Hester et al., 2006), and

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Abbreviations: EEG, electroencephalogram; ERP, event related potential; LED, light-emitting diodes; ANOVA, analysis of variance; CPP, conditioned place preference

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opiates (Lubman et al., 2000). This attraction has also been observed in addicted animals, which showed a preference for the drug-paired environment, for example, in the conditioned place preference (CPP) model. However, it remains unclear how drug-related cues attain their salience.

Previous studies have indicated strong physical reactions to drug-related environmental stimuli (Wikler, 1948; Eikelboom and Stewart, 1982; Geier et al., 2000; Solomon and Corbit, 1974; Siegel, 1975; Poulos and Cappell, 1991; Koob et al., 1997). Some work on opiates or cocaine addiction found that the conditioned responses were much like those induced by the drug itself, such as feeling high and pupillary constriction (Wikler, 1948; Eikelboom and Stewart, 1982). It was also reported that drug-related cues may elicit aversive reactions similar to responses underlying withdrawal such as dysphoria, anxiety, and tachycardia (Solomon and Corbit, 1974; Siegel, 1975; Poulos and Cappell, 1991; Koob et al., 1997). Most of the above studies focused on subjective or physiological responses to laboratory cues of drug use. However, the self-reported information from the addicts themselves is not always accurate and may be influenced by investigators' demands. In addition, the physiological indexes such as skin temperature, skin resistance and heart rate are not the direct evidence of emotional change and brain activity induced by drug cues. A study with non-subjective measures suggested that the responses induced by smoking-related stimuli were appetitive but not aversive (Geier et al., 2000). Moreover, brain-imaging studies indicated that drug-related environmental stimuli could enhance the activity in the mesolimbic reward system including the ventral tegmental area (VTA), the nucleus accumbens (NAc), and the hippocampus (Sell et al., 1999, 2000; Due et al., 2002; Heinz et al., 2004; David et al., 2005), a neural network that has been shown to respond to the drug itself. These findings suggested that drug-related stimuli might induce similar central activities as addictive drugs and acquire positive motivational properties and elicit approaching behaviors. In the present study, we tested this hypothesis by comparing the electroencephalographic (EEG) changes induced by morphine and morphine-related environment.

Previous studies on EEG activity and ERP revealed that exposure to drug-related stimuli such as videotape, objects, or pictures related to drug use in a laboratory setting gave rise to decreased EEG power (Bauer and Kranzler, 1994; Hersh et al., 1995; Liu et al., 1998), enhanced P3 (Herrmann et al., 2000, 2001), and slow positive waves (Franken, 2003, Franken et al., 2004). Most of these human studies used the stimuli that were created outside of the laboratory, and for some reasons, the stimuli may not have anything to do with the pharmacological effects of the drug. Few studies have ever addressed the EEG activity induced by a natural environment where drugs were previously used, and it is close to impossible to use naive humans to create drug cues. Actually, there was a human study that developed a model of preference conditioning in humans using smoking as the reinforcer. Preference for smoking was seen but EEG data were only recorded on one electrode and there were little conditioned EEG effects (Mucha et al., 1998). In fact, in their study, an instrumental conditioning procedure was used so that the cue functioned more through its indirect associations rather than direct associations with the reinforcer. In the present study, we used a classical conditioning animal model in which injections occurred before placement in the paired environment and the drug-related environment was directly paired with the euphoria induced by morphine. This protocol allowed for observation of the effects of simple conditional stimuli.

Although numerous EEG studies on acute morphine effects demonstrated that morphine could produce dose-dependent EEG slow-wave bursts (Goldstein and Aldunate, 1960; Mayo-Michelson and Young, 1993; Meng and Young, 1994) and an increase in the total power spectra (Stamidis and Young, 1992a,b; Mayo-Michelson and Young, 1993), little information was available about the power changes in different frequency bands and in different brain regions. The relationship between the EEG activities induced by the morphine-related environment and morphine itself also remained unclear. In this study, we used twelve scattered electrodes to record the spontaneous EEG activity in morphine-addicted rats while they were exposed to a previous drug-using environment in a CPP paradigm and examined the differences between cue-elicited EEG activity and those induced by acute morphine itself. Our analyses mainly focused on the topographic and spectral power changes in different frequency bands in an attempt to reveal the differences of the central mechanisms between these conditions.

2. Results

2.1. *Acute morphine-induced behavior and EEG changes*

2.1.1. *Dose-dependent effects on EEG activity*

Morphine injection rapidly produced low-frequency high-amplitude EEG waves with a frequency of less than 10 Hz and an amplitude of at least 80 mV. After injection of the higher doses of morphine (5, 10, and 20 mg/kg), all rats showed a behavioral awake but stupor that was characterized by muscle rigidity, exopthalmos, straub tail, and ptosis. The lower doses of morphine (0.1, 0.5, and 1 mg/kg) induced rare EEG slow-wave bursts, and the higher doses (5, 10 mg/kg) produced intermittent high-voltage slow-frequency bursts, superimposed over the low-voltage fast-frequency EEG. With the highest dose of morphine (20 mg/kg), the intermittent bursting developed into a more continuous high-voltage EEG activity (Fig. 1A). The duration of EEG slow-wave bursts was assessed from the channel data. The percentage of slow-wave bursts induced by each dose of morphine is shown in Fig. 1B. A one-way ANOVA revealed that the duration of high-voltage slow-wave EEG bursts in response to morphine was significantly different among all of the morphine doses [$F(6,34) = 26.78$, $p < 0.0001$], and the Student–Newman–Keul's posttests showed a significant increase of slow-wave bursts at the doses of 5 mg/kg ($p < 0.05$), 10 mg/kg ($p < 0.01$), and 20 mg/kg ($p < 0.001$), compared with saline injection. Pearson correlation analysis revealed a positive correlation between the duration of slow-wave bursts and the drug dose ($r = 0.9076$, $p < 0.0001$) (Fig. 1C).

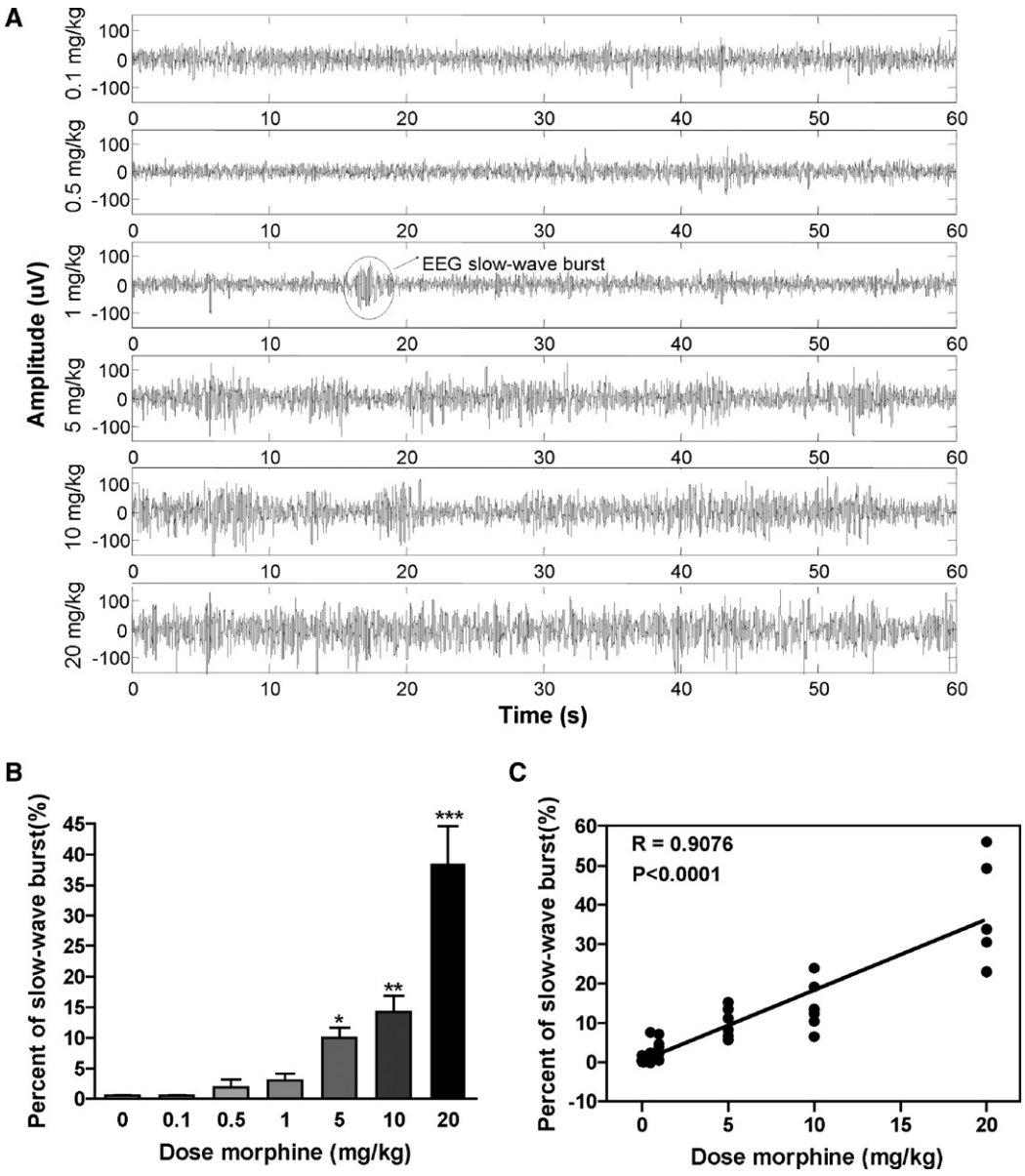

Fig. 1 – Morphine-induced EEG slow-wave bursts. (A) EEG activity recorded from lead F5 after morphine injection at a dose of 0.1, 0.5, 1, 5, 10, or 20 mg/kg. High-voltage EEG slow-wave bursts could be observed at the higher doses of morphine. (B) Effects of morphine as a function of doses on high-voltage slow-wave EEG burst duration. Data are presented as mean ± SEM (n = 5–6). *,: $p < 0.05$ and $p < 0.01$, respectively, compared with the saline control group. (C) Percentage of EEG slow-wave bursts showed a significant dose-dependent increase.**

2.1.2. Dynamic changes of EEG power in the time course of morphine administration

Based on the abovementioned results that morphine in the higher doses of 5–20 mg/kg but not the lower doses of 0.1–1 mg/kg induced significant slow-wave bursts, we analyzed the dynamic changes of EEG power in the 5 mg/kg dose group. As can be seen from the color-coded images of Z-scores (Fig. 2A), morphine administration caused an increase in the EEG power of slow (1.25–5 Hz) and middle rhythms (10–25 Hz), which started within 5 min after morphine injection, and was maintained across the whole recording period (50 min). In contrast, the EEG power of fast rhythms (>30 Hz) decreased following morphine administration. To quantify the dynamic changes in EEG power, we averaged the Z-scores in the 1.25–25 Hz and 30–45 Hz bands, respectively. As shown in Fig. 2B, the power in the low-frequency band (1.25–25 Hz, indicated by blue line) increased while the high-frequency band (30–45 Hz, green line) deceased following morphine injection. The total EEG power across the observed frequency range (1.25–45 Hz, red line) presented a trend of increase consistent with the low-frequency range.

2.1.3. Dose-dependent changes in EEG power

The spectral power density curve showed that, before morphine administration, EEG synchronized within a small range

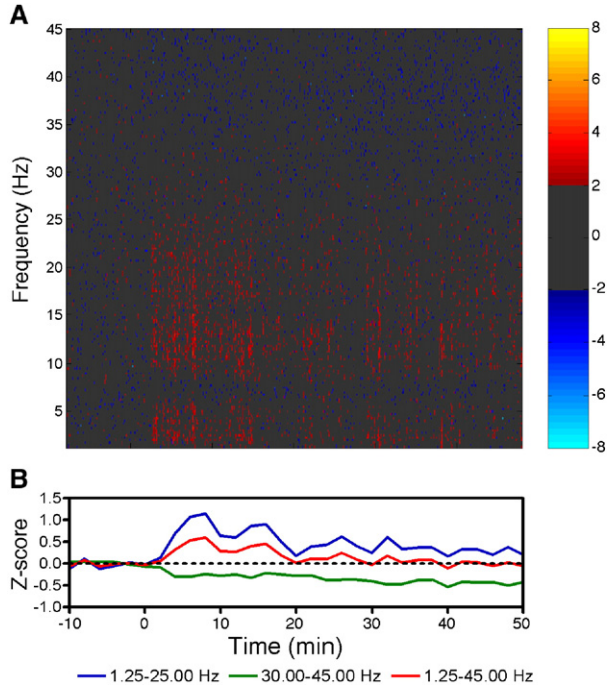

Fig. 2 – Dynamic changes of EEG power in the time course of morphine administration. Data of mean spectral power from lead FO4 in the 5 mg/kg dose group are shown. (A) Normalized time-based power spectrum density to the baseline level. Time = 0 on the *x*-axis corresponds to the time of morphine administration. Color-coded *Z*-scores are displayed (red for increase and blue for decrease). (B) Averaged *Z*-scores in the three frequency bands. After morphine administration, total power (red line) and the power in the 1.25–25 Hz band (blue line) gradually increased to its peak and kept above the baseline level for at least 40 min; on the contrary, the power in the 30–45 Hz band (green line) decreased after morphine administration.

of peak frequency which centered at 7.29 ± 0.06 Hz (n = 41). This peak of power was reduced (0.1–5 mg/kg dose) or eliminated (10–20 mg/kg dose) by morphine administration. Furthermore, morphine at the higher doses of 5, 10, and 20 mg/kg produced significant power increases in the slow-frequency range (1.25–5 Hz), with a mean absolute power change from 17.96 ± 0.60 dB during baseline to 19.71 ± 0.44 dB, from 18.38 ± 0.86 to 20.53 ± 0.69 dB, and from 16.02 ± 1.00 to 22.01 ± 1.91 dB, respectively (paired t-test, $p < 0.01$ for all the three doses). There were also significant increases in the middle frequency range (10–20 Hz) at the doses of 5 mg/kg (changed from 8.63 ± 0.20 to 10.26 ± 0.48 dB, $p < 0.01$), 10 mg/kg (changed from 8.55 ± 0.52 to 11.40 ± 0.54 dB, $p < 0.01$), and 20 mg/kg (changed from 6.76 ± 0.98 to 10.74 ± 1.22 dB, $p < 0.01$) (Fig. 3).

Further analyses of EEG power in each frequency band were performed using two-way repeated-measures ANOVAs. Although some treatment × leads interactions for higher doses of morphine were found (see Section 2.3), morphine injection induced changes toward the same direction across all of the leads in most of the frequency bands. A statistically significant main effect of treatment was found in each band (Table 1). As shown in Table 1, all doses of morphine, no matter low and high, produced qualitatively similar changes in absolute EEG power, characterized by an increase in the delta, theta, alpha1, alpha2, beta1, and beta2 bands and a decrease in the gamma band. More increases were found in the alpha2, alpha1, and beta1 bands with a mean percentage change (across all of the morphine doses) of 27.1%, 17.8%, and 14.9% respectively. Less increases were found in delta (12.0%), theta (8.2%), and beta2 (6.1%) bands. The highest dose of morphine (20 mg/kg) produced the most significant change (36.6%), and the lowest dose of morphine (0.1 mg/kg) only produced a change of 2.3%. By using Pearson correlation analysis, we found that total power was positively correlated with the drug dose ($r = 0.8954$, $p < 0.0001$) (Fig. 4, top left panel). In addition, the power in each frequency band also showed dose-dependent changes: a positive correlation in the delta ($r = 0.8663$, $p < 0.0001$), theta ($r = 0.8320$, $p < 0.0001$), alpha1 ($r = 0.8326$, $p < 0.0001$), alpha2 ($r = 0.8823$, $p < 0.0001$), beta1 ($r = 0.8918$, $p < 0.0001$), and beta2 ($r = 0.7957$, $p < 0.0001$) bands and a negative correlation in the gamma band ($r = -0.7046$, $p < 0.0001$) were found (Fig. 4).

The results of relative power showed a qualitatively similar change across all of the doses in most of the bands except the delta band (Table 1). The maximal relative power increase was found in alpha2 (13.6%). This could be inferred from the maximal absolute power increase in this band. Similarly, an increase in alpha1 (6.3%) and beta1 (3.6%) was detected. With lower increase (delta, theta, beta2) or a decrease (gamma) in absolute EEG power, a slight increase or a decrease in relative power was found in the delta (1.2%), theta (−2.2%), beta2 (−3.7%), and gamma (−11.95%) bands. These data showed that, relative to the increased total power, morphine injection increased the activities primarily in the alpha2, alpha1, and beta1 bands.

2.2. *Morphine-related environment-induced EEG activities after the induction of a positive place conditioning*

After conditioning the preference scores of rats in the morphine-paired group (0.571 ± 0.026) were significantly higher than those before conditioning (0.482 ± 0.023, $p < 0.05$). In contrast, there were no significant changes in the preference scores in the other two groups ($P > 0.05$).

In the preconditioning session, there were no significant differences in EEG spectral power density during exposure to the two CPP compartments (Fig. 5A). However, after CPP training, in the morphine-paired group, EEG power was significantly decreased in the frequency range from 9.75 to 15.5 Hz during presentation of the drug-paired compartment (10.07 ± 0.70 dB) when compared with the neutral one (8.83 ± 0.42 dB) (Fig. 5B). No significant differences in EEG power were found in the other two groups (the drug-unpaired and control group).

The results of two-way repeated measures ANOVAs revealed significant environment effects in the delta [$F(1,80) = 27.443$, $p < 0.0001$], theta [$F(1,80) = 52.216$, $p < 0.0001$], alpha1 [$F(1,80) = 14.032$, $p < 0.001$], alpha2 [$F(1,80) = 47.348$, $p < 0.0001$],

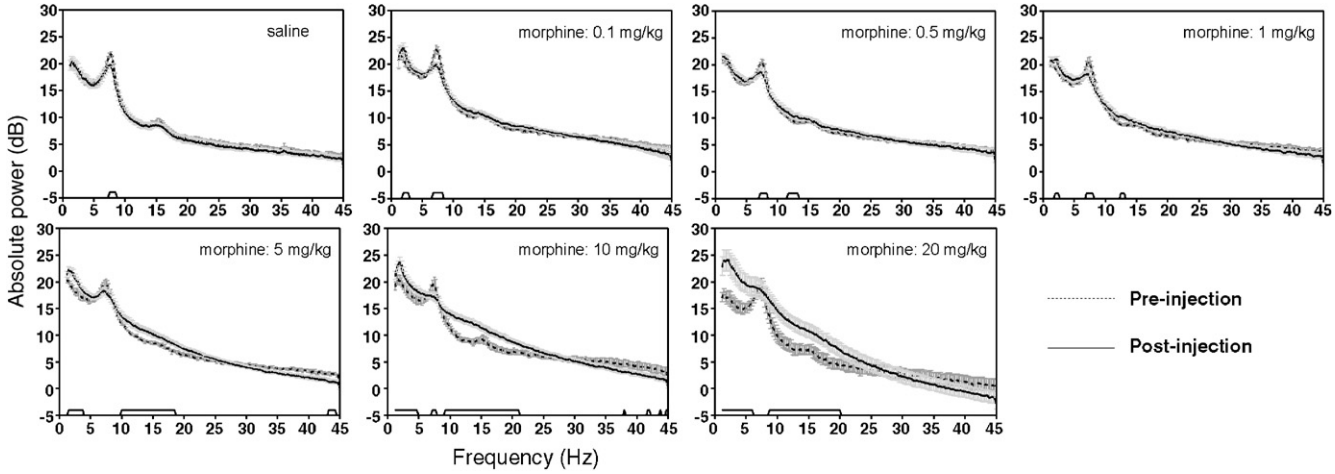

Fig. 3 – Morphine-induced EEG change in the frequency domain. Data were taken from lead F6. The mean and the SEM of spectral power density during baseline and post-drug are displayed. The trapezoid marker along the *x*-axis indicates the statistically significant difference between the spectral power before (dashed line and dark gray shadow) and after (solid line and light gray shadow) drug administration (paired *t*-test, $p<0.05$). Morphine administration induced a power increase in the ranges of 1–5 and 10–20 Hz.

beta1 [$F(1,80)=47.333$, $p<0.0001$], and beta2 [$F(1,80)=38.904$, $p<0.0001$] bands for the morphine-paired group, characterized by a lower absolute EEG power in the drug-paired compartment (Fig. 6A) than in the drug-unpaired compartment. Results of relative power showed that, after conditioning training, EEG power in the delta [$F(1,80)=8.905$, $p<0.01$], beta2 [$F(1,80)=44.33$, $p<0.0001$], and gamma [$F(1,80)=21.35$, $p<0.0001$] bands was significantly higher in the morphine-paired compartment relative to the neutral one, while the relative power in the theta [$F(1,80)=4.178$, $p<0.05$], alpha2 [$F(1,80)=34.34$, $p<0.0001$], and beta1 [$F(1,80)=32.09$, $p<0.0001$] bands was significantly lower in the morphine-paired compartment.

To determine if movement influenced the EEG activity, the time points of infrared beam crossings were recorded, and the movement speed was also calculated. The movement speed was evaluated at six levels: <6 ms/s, 6–12 ms/s, 12–17 ms/s, 17–25 ms/s, 25–50 ms/s, and >50 ms/s. Results of two-way repeated-measures ANOVA showed no significant differences in rats' movement when they were placed in the drug-paired and drug-unpaired compartment for all the three groups (Fig. 6B).

2.3. *Comparison between morphine- and cue-induced EEG activities*

From the scalp mapping of EEG power, it is evident that cue-induced change in absolute EEG power was drastically different from that induced by low (0.5 mg/kg) and high doses (5 mg/kg) of morphine (Fig. 7A). Visual inspection of the EEG topographic maps showed that, after morphine administration, delta power mainly increased in the centro-frontal region (F5 and F6). The maximal power change in the theta band occurred at the lateral electrodes (F7, F8,

Table 1 – Acute morphine injection-induced changes in absolute and relative EEG power

Dose (mg/kg)	Calculation	EEG power bands						
		delta	theta	alpha1	alpha2	beta1	beta2	gamma
0.1	absolute	3.2***	−0.7	3.2**	3.8***	2.5***	2.4***	−0.1
	relative	1.3**	−2.6***	1.4	1.9**	0.6**	0.5	−2.0***
0.5	absolute	3.3***	1.8**	3.9**	5.7***	3.8***	3.0***	0.4
	relative	0.3	−1.2**	0.9	2.6**	0.7*	0.1	−2.5***
1	absolute	6.4***	3.1**	8.4***	11.1***	5.9***	3.1***	−1.9***
	relative	1.6*	−1.5**	3.5**	6.0***	1.2***	−1.4*	−6.2***
5	absolute	11.3***	8.4***	17.9***	25.0***	14.8***	6.0***	−3.7***
	relative	0.5	−2.0***	6.9***	12.8***	3.7***	−4.1***	−12.8***
10	absolute	11.8***	10.6***	24.1***	38.6***	21.1***	7.2***	−5.5***
	relative	−1.6**	−2.5***	9.8***	22.1***	6.7***	−5.6***	−16.9***
20	absolute	35.9***	25.9***	49.3***	78.1***	41.0***	14.9***	−10.9**
	relative	4.8***	−3.1***	15.2***	35.9***	8.5***	−11.5***	−31.3***

Mean percentage change of the twelve leads was calculated for each morphine dose, and the differences between morphine injection and saline injection are shown (positive for increase and negative for decrease; *$p<0.05$, **$p<0.01$, ***$p<0.001$).

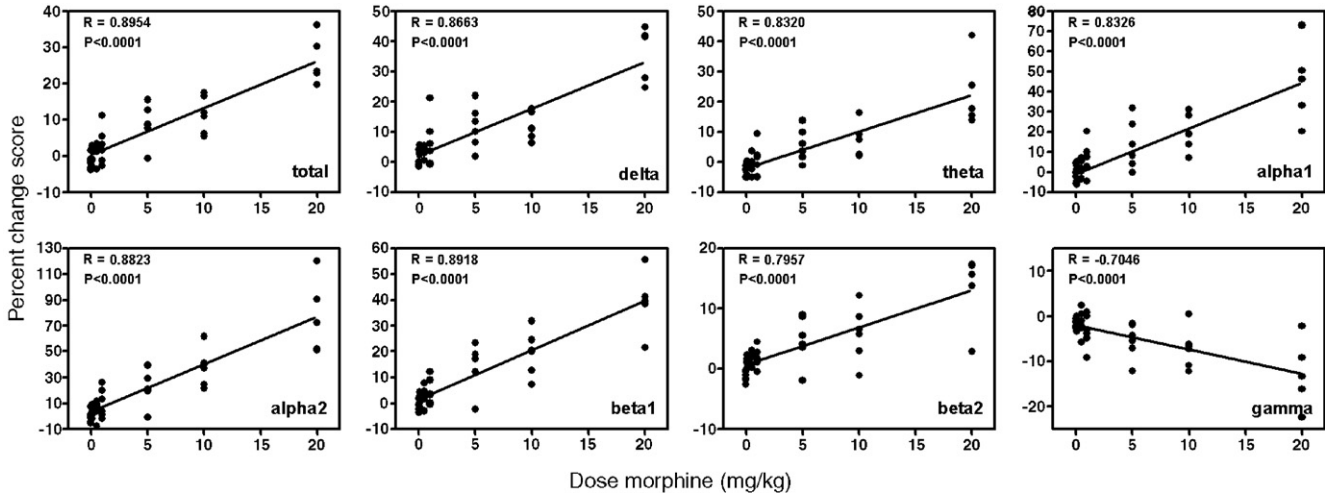

Fig. 4 – Dose–response effects of morphine on percentage change in absolute EEG power. Since all of the electrodes showed similar dose-dependent response, averaged data over all of the electrodes were calculated for each animal and these averaged values were plotted. A significant positive correlation was found in total frequency band (top left panel). Pearson correlation analysis also revealed a positive correlation in the delta, theta, alpha1, alpha2, and beta1 and beta2 bands, and a negative correlation in the gamma band.

FO3, and FO4). Alpha1 power changed mostly at the centro-frontal and lateral electrodes (F3, F4, F7, F8, FO3, and FO4) with higher doses (5–20 mg/kg). The power in the alpha2, beta1, beta2, and gamma bands exhibited a maximal change in the centro-frontal region (F3, F4, F5, and F6) when higher doses of morphine were given. Statistically, a significant treatment×lead interaction effect was found for the theta [$F(11,116)=3.283$, $p<0.001$] and alpha2 [$F(11,116)=1.883$, $p<0.05$] bands in the 10 mg/kg dose group. In the theta [$F(11,104)=2.833$, $p<0.01$], alpha1 [$F(11,104)=1.993$, $p<0.05$], and alpha2 [$F(11,104)=3.149$, $p=0.001$] bands, a significant treatment×lead interaction was also present in the 20 mg/kg dose group. These interaction effects suggest that the regional distinctions were related to morphine treatment. In contrast with morphine, the morphine-related environment induced a diffuse decrease in absolute EEG power without any regional difference (bottom panel in Fig. 7A). No environment×leads interaction effects were demonstrated.

Although the relative power showed different profiles of change from the absolute power after morphine injection, the regional distinctions were similar (top two panels in Fig. 7B). There were significant treatment×leads interactions for the theta [$F(11,104)=2.035$, $p<0.05$], alpha2 [$F(11,104)=2.798$, $p<0.01$], beta2 [$F(11,104)=2.044$, $p<0.05$], and gamma [$F(11,104)=4.017$, $p<0.0001$] bands in the

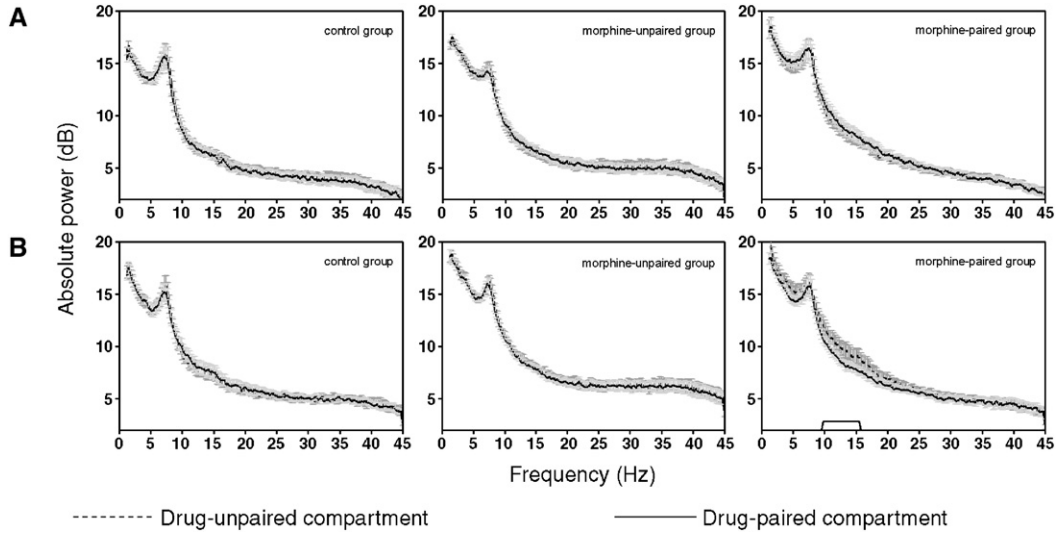

Fig. 5 – Differences in the absolute power from lead F6 in the two CPP compartments. Mean and SEM of power spectra density in the drug-paired compartment (solid line and light gray shadow) and drug-unpaired compartment (dashed line and dark gray shadow) are shown. Morphine-paired environment induced a general power decrease, and the change in the range from 9.75 Hz to 15.5 Hz was statistically significant (indicated with the trapezoid marker).

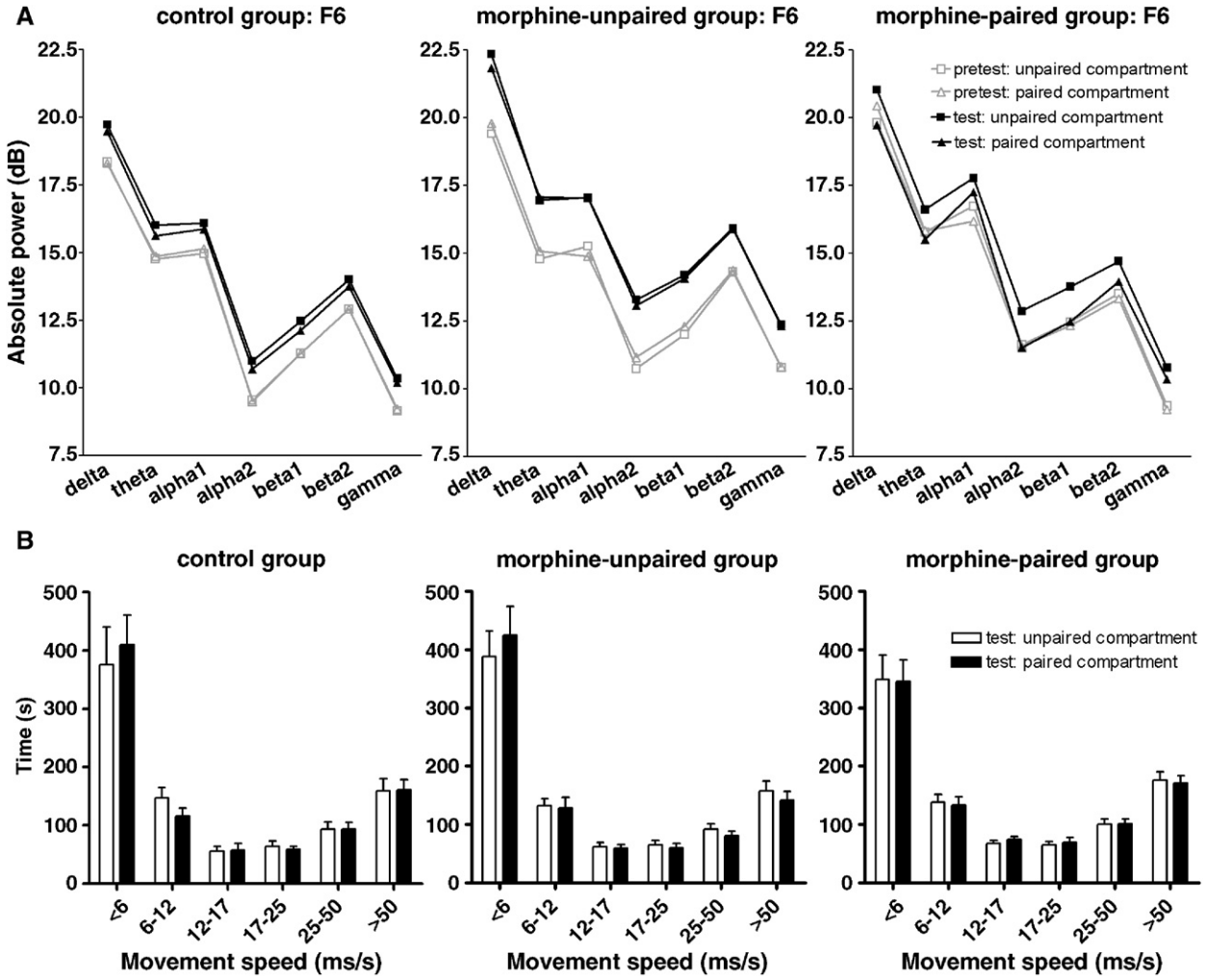

Fig. 6 – Comparisons of the absolute power and the movement speed when rats were exposed to the drug-paired compartment and the non-paired compartment. (A) In the morphine-paired group, absolute EEG power was significantly decreased during presentation of the drug-paired compartment when compared with the neutral one. No significant differences in EEG power were found in the other two groups. (B) Movement speeds were evaluated at six levels. No significant differences were found when rats were placed in the drug-paired compartment and drug-unpaired compartment on the postconditioning day.

20 mg/kg dose group, the alpha2 [$F(11,116)=2.268$, $p<0.05$], beta2 [$F(11,116)=2.306$, $p<0.05$], and gamma [$F(11,116)=4.003$, $p<0.0001$] bands in the 10 mg/kg dose group and the beta2 [$F(11,116)=1.878$, $p<0.05$] band in the 5 mg/kg dose group. Compared with morphine, morphine-related environment induced opposite topographic changes, especially in the alpha1, alpha2, beta1, beta2, and gamma bands (bottom panel in Fig. 7B). Although visual inspection showed that there was an increase in the delta, beta2, and gamma power at the centro-frontal region (F3 and F4), statistical analysis revealed no significant environment×lead interaction effects.

3. Discussion

The present study provided the first evidence that morphine and morphine-related environment induced opposite EEG power changes. Our results demonstrated that acute morphine administration resulted in EEG synchronization (an increase in power) while morphine-related environment led to diffuse EEG desynchronization (a decrease in power). These results indicate that the central mechanisms underlying the motivation of morphine place preference might be different from the psychic effects induced by acute morphine administration.

3.1. *Acute morphine administration-induced EEG changes*

Previous studies reported that morphine could produce biphasic EEG and behavioral responses. For example, Khazan and Colasanti (1971) reported that morphine administration (10 mg/kg) induced an initial phase of stupor lasting for 60–90 min followed by a 60–90 min period of arousal. During the stupor phase, behavioral stupor was accompanied by EEG slow-wave bursts (Goldstein and Aldunate, 1960; Mayo-Michelson and Young, 1993; Meng and Young, 1994). Similarly, we observed in the present study that morphine elicited dose-

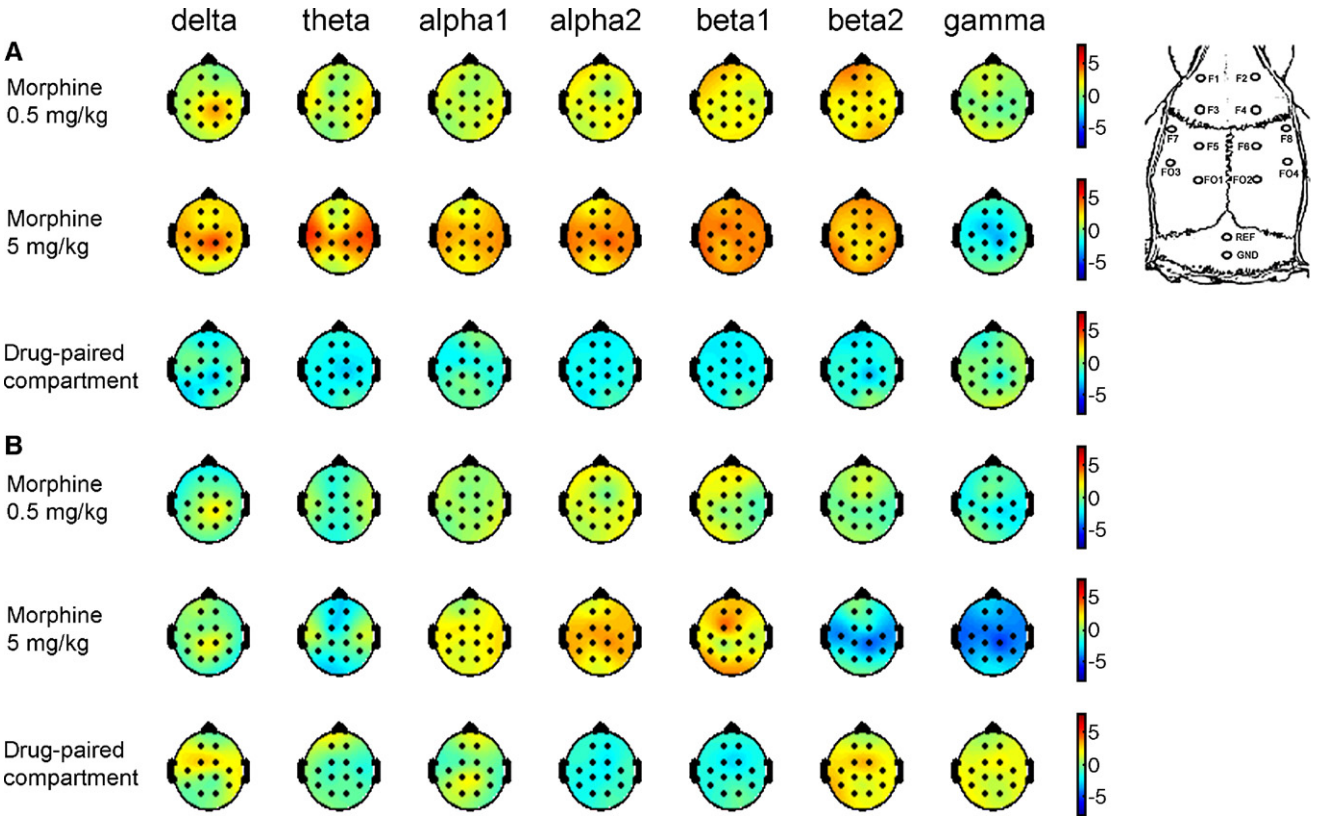

Fig. 7 – Topography of the changes in absolute and relative power induced by acute morphine administration and drug-related environment. Changes are displayed as color-coded *t*-values (red for increase and blue for decrease). (A) Changes in absolute power induced by morphine (0.5, 5 mg/kg) and drug-related environment. (B) Changes in relative EEG power induced by morphine (0.5, 5 mg/kg) and drug-related environment.

dependent EEG slow-wave bursts with a duration of 60–90 min. We also found that both low (0.1–5 mg/kg) and high (10–20 mg/kg) doses of morphine produced an increase in EEG power in the low and middle frequency bands and a decreased EEG power in the gamma band, which was consistent with other studies (Trampus et al., 1990; Meng and Young, 1994; Sun et al., 2006), although Ferger and Kuschinsky (1995) reported that a low dose (3 mg/kg) of morphine caused a general power decrease except in beta2.

In our study, morphine injection produced a maximal increase in EEG power in the alpha2 band. This observation may be related to the morphine-evoked euphoria. Human studies on alcohol- (Lukas et al., 1986a,b, 1989) and marihuana-induced euphoria (Lukas et al., 1995) have consistently demonstrated alpha enhancement. For instance, Lukas et al. (1986b) described that the increases in alpha activity paralleled the onset of alcohol-induced euphoria. Our topographic maps showed that the most pronounced increase in alpha activity was located in the centro-frontal region. Although it has long been accepted that alpha EEG wave which occurs during wakefulness originates largely from the posterior regions of the head (Niedermeyer, 1987), recent development of dipole source analysis revealed an anterior alpha activity situated in the anterior cingulated cortex (ACC) (Connemann et al., 2001; Connemann et al., 2005). Interestingly, Lukas et al. (1989) observed that the distribution of EEG alpha activity extended further frontally to the central sulcus during ethanol intoxication. Therefore, the increased alpha activity in the centro-frontal area in our study may be related to the euphoria induced by morphine. This view is further supported by the spatial distribution of opioid receptors. ACC is among the brain areas with highest densities of opioid receptors in the central nervous system (Vogt et al., 1995). Several studies have demonstrated that morphine or heroin could increase the activity in the ACC, the medial prefrontal cortices, and the other limbic structures (Jones et al., 1991; Chang et al., 1997, 1998; Shah et al., 2005). A SPECT study demonstrated that the mu opioid agonist hydromorphone produced good subjective effects and was accompanied by an increased regional cerebral blood flow (CBF) in these structures (Schlaepfer et al., 1998). Considering the superficial position of the ACC (Cardinal et al., 2003), the markedly increased alpha activity at the centro-frontal region in this study may come from ACC. Moreover, from the topographic maps plotted with *t*-values, we found that the increase in the alpha band at the centro-frontal cortex did not show a dose-dependent relationship. It was most significant with a dose of 10 mg/kg (data not shown). This may be associated with the activation of low affinity kappa receptors by large doses of morphine (20 mg/kg), which can reduce morphine reward (Walters et al., 2005).

Another finding in this study was the shift of synchronization from within the small window of peak frequency around 7.29 Hz to a broader frequency band. According to Klimesch (1996), the EEG theta activity may be generated from the hippocampus via hippocampo-neocortical feedback loops. Many studies corroborated that opiates were effective in attenuating hippocampal theta activity (Khanna, 1997; Khanna and Zheng, 1998; Zheng and Khanna, 1999) and that these changes may be detected by scalp electrodes.

3.2. *Cue-induced EEG changes*

In the present study we successfully established CPP with morphine at a dose of 5 mg/kg for behavioral observation and EEG recording. In the postconditioning test, animals were confined alternatively in the two CPP compartments associated with morphine injection or saline injection under a drug-free condition. In this model, we compared the EEG activity between the drug-related environment and the neutral environment. For the morphine-paired group, morphine-related environment produced a general decrease of power in all of the frequency bands except gamma, while the rats' movements in these two environments did not show any differences. These results were consistent with those reported in cocaine users (Bauer and Kranzler, 1994; Hersh et al., 1995; Liu et al., 1998) in which drug-related cues produced diffuse decreases in EEG power. The authors attributed the results to cue-induced cortical arousal. In fact, place conditioning can be viewed as a Pavlovian incentive response. As a result of the association with the unconditioned stimuli, the representation of the conditioned environment acquired the ability of inducing preparatory-incentive responses, which are commonly attributed to a state of motivational arousal (incentive arousal) (Di Chiara, 2002). On the other hand, in Franken's (2003) review, sufficient evidence was cited to demonstrate that these changes in the background EEG were related to changes in the attentional state. Gomez et al. (2004) found that during expectant periods there was a general decrease in the power spectral density between 0 and 42.9 Hz. These results suggest a state of expectancy for reward when the CPP rats were in the drug-paired environment. It is possible that the anticipatory behavior of the rats is reflected in an increased arousal and increased attention towards the drug-related stimuli.

3.3. *Comparison between morphine- and cue-induced EEG activities*

The EEG changes elicited by morphine-related environment were obviously different from those induced by morphine itself. Nieto et al. (2002) reported that, when rats were placed in the morphine-paired environment, the extracellular level of enkephalin in the NAc was increased within 30 min. This suggested that the morphine-related environment might to some extent be able to simulate the central effects of morphine. Following this hypothesis, the brain activities related with morphine-paired environment and morphine itself should be somehow similar. However, our current results showed that the EEG activity elicited by environmental cue was obviously different from that by morphine itself. This was still true even after extending the dose of morphine to a lower range of 0.1–1.0 mg/kg, in an attempt to mimic the level of endogenous opioids in brain tissues. In our study, all doses of morphine, no matter how low (0.1 mg/kg) or high (20 mg/kg), produced qualitatively similar effects of an increase in EEG power, which was opposite to the results produced by drug-related environment, i.e., a decrease in EEG power. These differences may be explained by the fact that EEG activity is more likely to be influenced by the activities of cortex rather than by deeper parts of the brain such as the NAc.

Several possible explanations could be issued for our observed opposite EEG changes induced by morphine-related environmental stimuli and morphine itself. Opponent-process theory (Solomon and Corbit, 1974) might play an important role in our observed phenomena. According to this theory, after some pairings of the conditioned stimuli and the pharmacological effects of a drug, the drug-compensatory responses were elicited as conditional responses. The presence of a conditioned stimulus may result in counteractive physiological changes, which minimize the drug effects predicted by the conditioned stimuli to maintain an organism's homeostasis (Sell et al., 2000). Therefore, rats are more tolerant to a drug when it is administered in the usual drug-paired environment than when it is administered elsewhere (Poulos and Cappell, 1991; Siegel et al., 2000). Similarly, rats display more behavioral withdrawal symptoms when, in the absence of the drug, they are placed in the drug-paired environment than when they are placed in an alternative environment (see review by Siegel and Ramos, 2002). In our study, even if the drug-paired environment did induce a drug-like effect, it may not have shown any physiological reaction due to the existence of tolerance. On the other hand, the conditioned counteractive physiological changes induced by drug-related cue may be expressed as "withdrawal symptoms", which may contribute to the opponent EEG changes compared with that induced by morphine. An important doubt for this hypothesis is that, if these compensatory conditional responses really occurred, the animals would have chosen to avoid the drug-paired cue rather than to approach it. Thus, it may not be proper to explain the observed phenomena using drug-compensatory effects.

Another factor that should be considered is sensitization. It has been suggested that sensitization was much easier to develop when morphine was given in association with a distinct and relatively novel test environment than when given in a physically identical environment in which they lived (Badiani et al., 2000). In the present study, before the EEG recording on the postconditioning day, CPP rats have received four repeated injections of morphine in the morphine-paired compartment, which was a novel environment different from their home cage. On the postconditioning day, a sensitization to morphine might have been developed. But in the experiment of acute morphine administration, EEG signals were recorded when morphine was injected the first time. It was possible that the sensitization of CPP rats may contribute to the different EEG changes induced by drug-paired environment and the

drug itself. However, in the present study, higher doses of morphine (5–20 mg/kg) were used, and the EEG changes produced by drug-related environment were totally different from those induced by these higher doses of morphine. These data indicate that the finding in our study cannot be explained by sensitization.

In addition to sensitization, novel environment has also been reported to increase drug effects (Ferguson et al., 2004). In our study, before acute morphine administration, rats received acclimatization in the testing environment four times, and for the CPP rats, there was a pretest and four times of conditioning training in the drug-related environment. This protocol made it possible for the EEG recording environments during the two experiments to be presented a similar number of times before the effects of acute morphine and morphine-related environment were tested, so that the possible influence of recording environments could be ruled out.

In conclusion, our current results found that morphine and morphine-related environment produced opposite EEG power changes, which does not support the hypothesis that morphine-related environment and morphine share similar central mechanisms. Further study of single-unit activities from deep brain areas may be necessary to clarify its mechanisms.

4. Experimental procedures

4.1. Animals

Ninety male Sprague–Dawley rats (provided by the Institute of Animal Research, Chinese Academy of Science, Beijing) aged 12 weeks were used, weighing approximately 250–280 g at the start of the experiment. They were housed four per cage with the temperature maintained at 22±1 °C and kept under a 12:12-h light/dark cycle (lights on at 8:00 am). Food and water were available *ad libitum*. Rats were habituated to the environment and subjected to daily handling for a week before the surgery. All experiments were carried out in accordance with the National Institute of Health *Guide for the Care and Use of Laboratory Animals* (NIH Publications No. 80-23) revised in 1996. Formal approval to conduct the experiments described has been obtained from the Committee on Animal Care and Use of the Peking University. All efforts were made to minimize the number of animals used and their suffering.

4.2. Drugs

Morphine hydrochloride powder was purchased from the Pharmaceutical Factory of Qinghai (China) and dissolved in sterile saline to its final concentrations. Both morphine and saline were administered intraperitonealy.

4.3. EEG electrode implantation

Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and mounted in a Kopf stereotaxic apparatus. After exposing the skull, 14 epidural electrodes (stainless steel screws, tip diameter 1 mm, impedance 300–350 Ω) were implanted bilaterally over the following skull regions (Shaw et al., 1999): anterior frontal (F1, F2), anterior (A) +4.5 mm, lateral (L) ±1.5 mm; centro-frontal (F3, F4, F5, F6), A ±1.5 mm, L ±4.5 mm; lateral frontal (F7, F8), A 0.0 mm, L ±4.5 mm; fronto-occipital (FO1, FO2, FO3, FO4), A −4.5 mm, L ±1.5 mm for FO1, FO2, and A-3.0 mm, L ±4.5 mm for FO3, FO4. The reference and ground electrode were positioned 2 mm and 4 mm caudal to lambda, respectively. Each electrode was connected to a socket and fixed to the skull with dental cement. Animals were administered penicillin (60,000 U, i.m.) before surgery to prevent infection and housed individually after surgery.

4.4. Apparatus

The place conditioning apparatus was a polyvinyl-chloride plastic rectangular box (75×22×30 cm), containing three black-colored conditioning compartments separated by guillotine doors. The two larger compartments (A and C, 30×22×30 cm) were separated by a small gray-colored center choice compartment (B, 15×22×30 cm). Compartment A had four light-emitting diodes (LEDs) forming a square on the wall and a stainless-steel mesh floor (1.3×1.3 cm^2) coated with plastic. Compartment C had four LEDs forming a triangle on the wall and a stainless-steel rod floor (1.3 cm apart) coated with plastic. Compartment B had a plain floor (Shi et al., 2003). Fifteen infrared beams spaced 5 cm apart were monitoring the motion of the rat. The infrared sensors communicated to a computer every 100 ms through an interface. In addition, there was an alternate environment for control purpose, i.e., a transparent plastic cage (D, 30×30×30 cm) with a plain floor and plain walls, and distinct from the home cage. This control environment was also used to record EEG when acute morphine effect was studied. All of the experiments were performed in darkness with dim lights for illumination.

4.5. Acute morphine administration

Forty-two rats were used in this experiment. One week following recovery from surgery, rats were placed in the control box (D) for acclimatization for 1 h, twice a day for 2 days. Experiments were conducted on the third day. Rats were randomly separated into 7 groups (n=6 for each group), and each group received saline or different doses of morphine (0.1, 0.5, 1, 5, 10, 20 mg/kg) injection, respectively. EEG signals were collected continuously beginning 10 min prior to morphine or saline injection and ending 50–100 min after the injection. For the 20 mg/kg dose group, only five rats were used for testing since one rat had a problem with the reference electrode and was thus excluded.

4.6. Place conditioning procedure

An additional 24 animals were used to examine cue-related EEG activity. The animals were randomly separated into three groups, namely, morphine-paired, morphine-unpaired, and control group (n=8 for each group). The procedure consisted of three phases: preconditioning, conditioning and postconditioning (Fig. 8). In the preconditioning test, rats were first

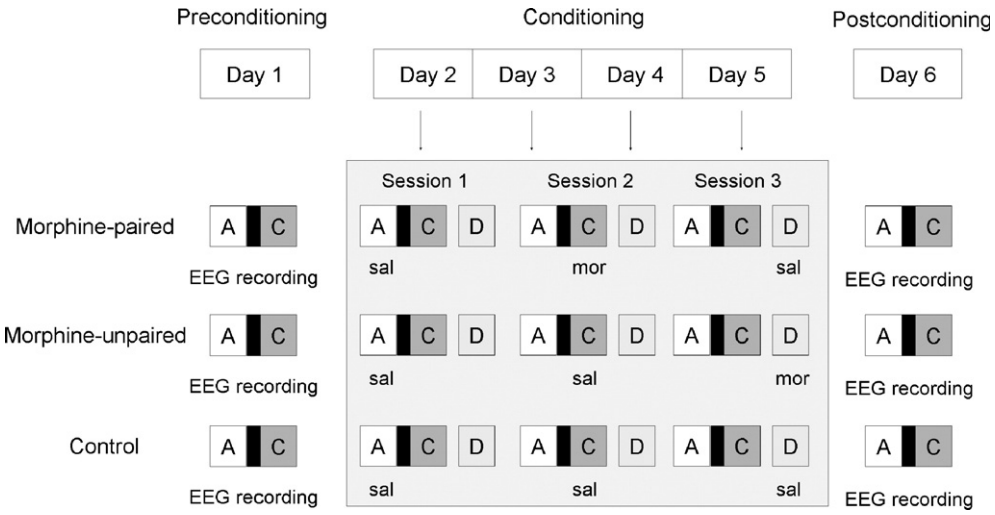

Fig. 8 – Schematic representation of CPP procedure. On the preconditioning day, all animals were put into the two CPP compartments for EEG recording. On the following 4 days, three injections per day were given paired with the two CPP compartments (A or C) or the control environment (D), respectively. The order of the injection and environment was counterbalanced across subjects. One pattern of the drug treatment and paired environment is shown for each group. On the postconditioning day, all animals were put into the two CPP compartments again for EEG recording.

placed in either of the CPP compartments (A or C) for a 15-min EEG recording, then in the home cage for 15 min, and finally transferred into the other CPP compartment (C or A) for another 15-min EEG recording. A counterbalanced design was used for the test order. On the following 4 days (i.e., conditioning training phase), rats received three sessions of conditioning training every day. Three injections (one morphine injection at a dose of 5 mg/kg and two saline injections) were given in the three sessions, at an interval of 6 h. After injection, rats were immediately confined to one of the three environments (A, C, and D) for 45 min. The morphine-paired animals received morphine injections before placement in one of the CPP compartments (A or C) and received saline injections before placement in the other CPP compartment (C or A) and the control box D. For the morphine-unpaired group, which served as a control for morphine treatment itself (Miller and Marshall, 2004), morphine injections were paired with box D and saline injections were paired with the two CPP compartments (A and C). The animals of the control group received saline injections in all of the three environments (A, C, and D). The order of the injection (morphine or saline) and compartment (A or C) was counterbalanced across subjects. On the following postconditioning day, EEG signals were recorded using the same paradigm as in the preconditioning test.

It was not possible to quantify the behavior with rats equipped with the cable for EEG recording. Therefore, a control experiment was conducted with 24 rats without electrode implantation for assessment of positive place conditioning. The protocol was the same as described above: (1) preconditioning phase, in which drug naive rats had free access to both compartments of the apparatus for a 15-min session; (2) conditioning phase, consisting of 4 consecutive conditioning days; (3) postconditioning phase, achieved 24 h after the last conditioning session and identical to the preconditioning session. The time spent in each compartment was recorded. Results are expressed in preference scores calculated as a ratio of the time spent in the drug-associated compartment to the total time spent in both compartments.

4.7. *Spectral analysis of EEG activity*

EEG data were digitized at a sampling rate of 256 Hz, re-referenced to the average, and passed through a 1.25–45 Hz bandpass filter. Some leads were eliminated due to noise or obvious artifacts. The digitized EEG data were segmented into 1.024-s epochs (50% overlap) and FFTs (Welch tapered window) were performed. Since the power spectral data (measured in μV^2/Hz) were significantly different from a normal distribution, these data were then normalized using a log transformation and expressed in dB. Absolute EEG power was obtained by compressing these transformed data into 7 frequency bands, which were defined as follows (Ferger and Kuschinsky, 1995): delta (1.25–4.5 Hz), theta (4.75–6.75 Hz), alpha1 (7–9.5 Hz), alpha2 (9.75–12.5 Hz), beta1 (12.75–18.5 Hz), beta2 (18.75–35 Hz), and gamma (35.25–45 Hz). Relative power (percentage of total power) for each frequency band was also calculated. In light of the individual differences of EEG power during baseline, the change in the EEG power after morphine administration was expressed as a percentage change from the baseline.

To visualize the dynamic changes in EEG power during morphine administration, time-based spectral power was computed using Matlab software with a frequency resolution of 0.25 Hz and a time resolution of 2 s. In order to find out the changes induced by morphine administration relative to the baseline better, the power spectra were normalized to the pre-administration baseline level using a Z-score transformation. Z-scores are a special application of the transformation rules. The Z-score can indicate how far and in what direction an item deviates from the mean value of its distribution,

expressed as $Z=(x-m)/s$, where x is the raw power spectra, m and s are the mean and standard deviation of the baseline, respectively. After Z-score transformation, the transformed scores will necessarily have a mean of zero and a standard deviation of one. The Z-score transformation is especially useful when seeking to compare the relative standings of items from distributions with different means and/or different standard deviations. After Z-score transformation, these values were then displayed as color-coded images and the Z-scores were also averaged along the whole time range.

4.8. *Statistical analysis*

For the power spectral data of acute morphine administration, a paired *t*-test was used to test at which frequency points the change of the absolute EEG power during post-morphine was significant with respect to the baseline. For the data in each frequency band, the percent change data were analyzed using a two-way repeated measures analysis of variance (ANOVA), with dose (a dose of morphine and saline) and leads (12 recording electrodes) as repeated measures. Bonferroni post-tests were used to compare the differences in each lead and the *t*-values were displayed as topographic color-coded maps by EEGLAB. Pearson correlations were used to assess relationships between changes in absolute EEG power and morphine dose.

Similarly, for the CPP data, a paired *t*-test and a two-way repeated-measures ANOVA (factors: environment and leads) were also applied to test the differences of power spectral density array and the EEG power (absolute or relative) in each band between the drug-paired environment and the non-paired environment, respectively. The differences in absolute and relative power between the two CPP compartments were indicated by *t*-values of Bonferroni posttests and displayed as colored scalp mappings. A two-way repeated-measures ANOVA was also used to compare rats' movement speeds when they were in the two CPP compartments (factors: environment and speed levels). A paired *t*-test was used to compare the preference scores between the preconditioning test and the postconditioning test for each group. Statistical analyses were performed with software GraphPad Prism 4.0. Results were presented as mean ± SEM.

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