

## Full-length article

## Endogenous histamine inhibits the development of morphine-induced conditioned place preference<sup>1</sup>

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### Key words

conditioned place preference; histamine; morphine; rat; tuberomammillary nucleus

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### Abstract

**Aim:** The conditioned place preference (CPP) paradigm was used to investigate the effects of endogenous histamine on the processes leading to morphine-induced reward-seeking behavior in Sprague-Dawley rats. **Methods:** The model of CPP was used to assess the rewarding effect of morphine. The levels of histamine, glutamate,  $\gamma$ -aminobutyric acid (GABA), dopamine (DA) and 3, 4-dihydroxyphenylacetic acid (DOPAC) in rat brains were measured with high-performance liquid chromatography. Immunohistochemistry technique was used to observe the morphological changes of neurons. **Results:** Intraperitoneal injection of morphine (2, 5 or 10 mg/kg) induced the development of CPP in a dose-dependent manner. In addition, morphine administrations (10 mg/kg) decreased the histamine content and reduced the number and size of histaminergic neurons in the tuberomammillary nucleus (TM), as well as markedly increasing the DOPAC/DA ratios in the ventral tegmental area (VTA) and nucleus accumbens (NAc). Intraperitoneal injection of histidine (50, 100 or 200 mg/kg) dose-dependently inhibited the development of morphine-induced CPP. Bilateral lesions of the TM, which decreased the histamine levels in the VTA and NAc, potentiated the development of CPP induced by morphine (1 mg/kg, a dose that produced no appreciable effect when given alone) and increased the DOPAC/DA ratios in the VTA and NAc, but did not change the glutamate or GABA levels in these nuclei. Histidine reversed the effects of the TM lesions. **Conclusion:** These results indicate that endogenous histamine plays a role in inhibiting the development of morphine-induced reward-seeking behavior, and the inhibition may involve the modulation of dopaminergic activity.

### Introduction

Morphine remains one of the most valuable drugs for the treatment of severe pain despite a large number of adverse side effects. The major drawback of their continuous use is the development of dependence, which is ascribed to their rewarding effects. These effects have been demonstrated in animals by the conditioned place preference (CPP) paradigm<sup>[1,2]</sup>, which is based on the principle that, when a primary reinforcer is paired with a contextual stimulus, the contextual stimulus itself acquires secondary reinforcing properties. These secondary reinforcing properties can elicit place

preference, which results in a significant increase in the time spent in the drug-paired place. The sensitivity of the CPP paradigm appears to be as great as or greater than those of other behavioral tests used to measure the rewarding effects of drugs<sup>[3]</sup>. However, in most studies to date, CPP is only tested after several conditionings of drugs paired with contextual stimulus. The process by which morphine-induced CPP develops is less well understood.

Histamine plays important roles as neurotransmitter and neuromodulator in the mammalian brain. Through its H<sub>1</sub>, H<sub>2</sub> and H<sub>3</sub> receptors, histamine participates in many physiological and behavioral functions including the sleep-wake cycle,



conjugated secondary antibody (goat anti-mouse antibody; Molecular Probes, Eugene, OR, USA) diluted 1:500 in PBS. The sections were then washed 3 times, mounted and examined under a fluorescence microscope (Olympus, BX51, Tokyo, Japan).

Neuron counting and measurements of perimeters were performed in sections from the saline- and morphine-treated brains. The outline of each HDC-positive neuron, together with the outline of the TM region, was drawn from all sections per brain. The number of HDC-positive neurons per section in each TM region was determined. The numbers of neurons derived from sections through the same brain were summed and divided by the number of sections. The perimeter of a HDC-positive neuron was measured using MetaMorph image analysis software (Universal Imaging Corporation, Downingtown, PA, USA). The length of the line outlining the neuron was used to assess the perimeter of HDC-positive neurons.

#### **Measurements of histamine, glutamate, $\gamma$ -aminobutyric acid (GABA), dopamine (DA) and 3,4-dihydroxyphenylacetic acid (DOPAC)**

**Sample preparation** The rats were decapitated 30 min after the final CPP test. The brains were quickly removed, placed on an ice-cold stainless steel plate, and the VTA and NAc were dissected according to the atlas of Paxinos and Watson<sup>[13]</sup>. The tissues were stored at -80 °C until assayed, when the samples were homogenized in 3% perchloric acid containing 5 mmol/L disodium ethylenediaminetetraacetic acid (EDTA) and 5-hydro-*N*<sup>o</sup>-methyltryptamine in a Polytron homogenizer (Kinematica, Lucerne, Switzerland) at the maximum setting for 20 s in an ice bath. The homogenate was centrifuged at 15 000×*g* for 20 min at 4 °C, then the supernatant was removed and filtered through a 0.22  $\mu$ m polyvinylidene difluoride membrane.

**Histamine, glutamate and GABA chromatography** Histamine levels were analyzed by HPLC combined with electrochemical detection according to our previously published method<sup>[14]</sup>. In brief, the system consisted of a model 582 pump, a model 540 autosampler and a 4-channel CoulArray electrochemical detector. The HPLC was controlled and the data acquired and analyzed using CoulArray<sup>®</sup> software. All equipment was from ESA Biosciences Inc (Chelmsford, MA, USA). After reacting with the derivative *o*-phthalaldehyde, analytes were separated on a 3  $\mu$ m, 3 mm×50 mm Capcell Pak MG C18 column (Shiseido, Tokyo, Japan). A two-component gradient elution system was used, with component A of the mobile phase being 100 mmol/L Na<sub>2</sub>HPO<sub>4</sub>, 13% acetonitrile, and 22% methanol (pH 6.8) and component B being similar, but with 5.6% acetonitrile and 9.4% methanol. The

gradient elution profile was: 0–3.5 min, isocratic 100% B; 3.5–20 min, linear ramp to 0% B; 20–22 min, isocratic 0% B; 22–23 min, linear ramp to 100% B; and 23–30 min, isocratic 100% B (flow rate 0.75 mL/min). The first cell was set at +250 mV and the second at +350 mV. All standards were obtained from Sigma (USA). Under these conditions, the retention times of glutamate, GABA and histamine were 5.58, 15.16, and 18.36 min, respectively. The detection limits (signal/noise  $\geq$ 3) were 0.1  $\mu$ g for glutamate, 5 ng for GABA, and 1 ng for histamine. Values were expressed as nmol/g wet tissue.

**DA and DOPAC chromatography** DA and DOPAC levels were determined with the above HPLC system, using methods previously described<sup>[15]</sup>. The mobile phase consisted of 71.5 mmol/L citric acid, 78.3 mmol/L citric acid trisodium salt, 832  $\mu$ mol/L 1-octanesulfonic acid sodium salt, 107  $\mu$ mol/L EDTA, pH 5.6 with 6% methanol. DA was separated on a reverse-phase column (HR-80, 4.6 mm×80 mm, 3  $\mu$ m, ESA, USA) at 30 °C and quantified by a 4-channel CoulArray electrochemical detector. The cell potentials were E1, -5 mV, and E2, +220 mV (flow rate 0.60 mL/min). DA and DOPAC levels were expressed as nmol/g wet tissue.

**Surgical procedures** Under chloral hydrate anesthesia (360 mg/kg, ip), the rats were fixed in a stereotaxic frame (Narishige, SR-5, Tokyo, Japan), and bipolar stainless steel electrodes (tip diameter 0.2 mm) were implanted bilaterally into the TM (AP -4.2 mm, L  $\pm$ 1.5 mm, and DV -9.6 mm). An electrolytic lesion was made by applying 1 mA DC for 10 s. In the case of a sham lesion, the electrodes were implanted into the same locations and connected to the lesion device, but no current was passed. At the end of the experiments, the animals were sacrificed and the locations of the electrodes were verified histologically. Only animals with electrodes located in the TM were included in the subsequent analysis.

**Drugs** Morphine hydrochloride (First Pharmaceutical Factory of Shenyang, Shenyang, China) and histidine hydrochloride (Sigma, USA) were dissolved in saline. Histamine, glutamate, GABA, DA and DOPAC were purchased from Sigma (USA).

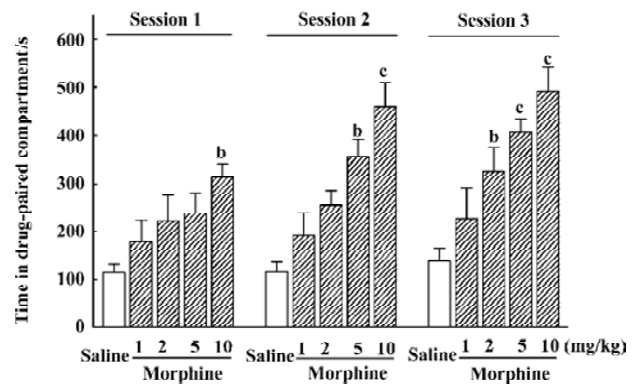
**Statistical analysis** Data were analyzed with GraphPad Prism 4 (GraphPad Software Inc, San Diego CA, USA) and expressed as the mean $\pm$ SEM. Place preference data were evaluated by two-way ANOVA followed by the Bonferroni test as a post test. Neurochemical data were evaluated by one factor ANOVA, followed by the Bonferroni test as a post test. Differences in histamine levels, DOPAC/DA ratios and the numbers and perimeters of HDC-positive histaminergic neurons between the saline- and morphine-treated groups were analyzed by Student's *t*-test. Statistical significance

was set at  $P < 0.05$ .

## Results

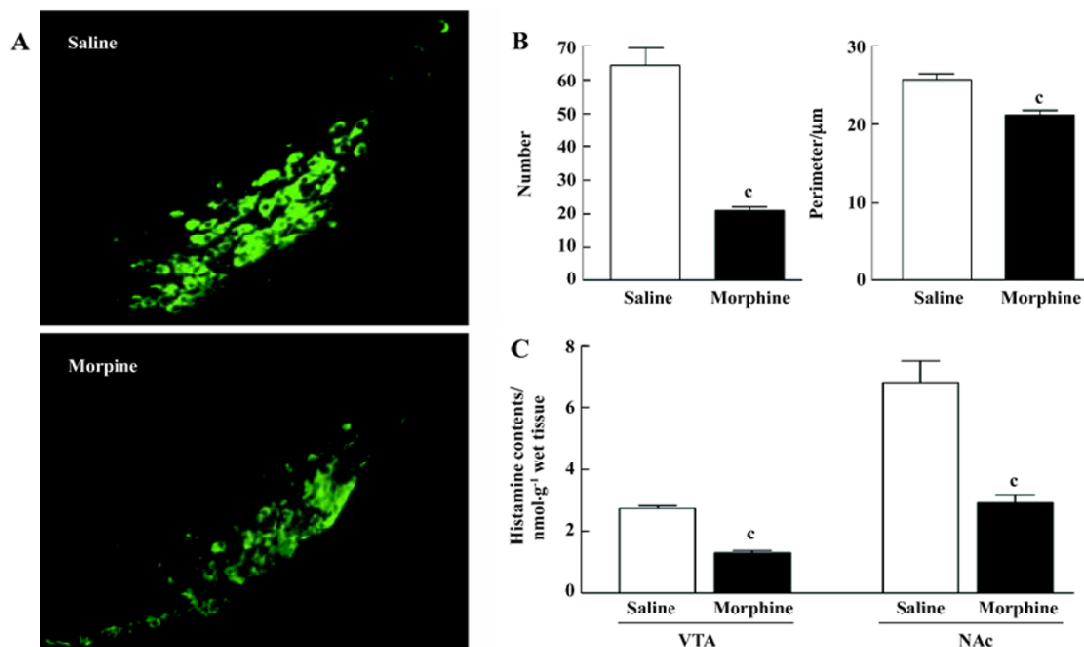
**Effect of morphine on the development of CPP** In the baseline preference testing, animals showed a preference for the black compartment, where they spent  $831.59 \pm 21.31$  s. CPP was affected by the morphine dose ( $F_{4,154} = 10.81$ ;  $P < 0.01$ ) and the number of conditioning sessions ( $F_{2,154} = 3.69$ ;  $P < 0.05$ ), but there was no interaction between these variables (Figure 2). An analysis of each conditioning session revealed that the rats treated with 2 mg/kg morphine showed significant CPP relative to the saline-treated group at its third conditioning session; 5 mg/kg morphine had similar effects at the second and third conditioning sessions, and a dose of 10 mg/kg showed significant CPP after the first treatment.

**Effect of morphine on histamine levels in the VTA and NAc, and morphology of histaminergic neurons in the TM** After 10 mg/kg morphine administrations, histamine levels in the VTA and NAc significantly decreased (VTA:  $P < 0.01$ ; NAc:  $P < 0.01$ ; Figure 3C). Histamine levels decreased to 47.4% in the saline-treated group in the VTA and to 43.0% in the saline-treated group in the NAc. In addition, green fluorescent protein-tagged HDC-positive (histaminergic) neurons



**Figure 2.** Effect of morphine on the development of CPP in rats. Morphine was injected ip 5 min before conditioning ( $n = 14-16$ ). Data are mean  $\pm$  SEM of time spent in the drug-paired compartment. <sup>b</sup> $P < 0.05$ , <sup>c</sup> $P < 0.01$  vs saline-treated group during same conditioning session (ANOVA followed by Bonferroni test).

exhibited considerable variability in shape compared with those from the saline-treated rats. Furthermore, HDC-positive neurons were smaller and sparser in the morphine-treated rats (Figure 3A). An analysis of the HDC-positive neurons from the saline- and morphine-treated rats revealed that morphine reduced both their mean number and size (Figure 3B).



**Figure 3.** Effects of morphine (10 mg/kg) on histamine levels in the VTA and NAc and morphology of histaminergic neurons in the TM. (A) Morphology of HDC-positive histaminergic neurons in sections through TM from saline-treated and morphine-treated animals; (B) Number and perimeter of HDC-positive histaminergic neurons in sections through TM. Data are mean  $\pm$  SEM of 8 observations. <sup>c</sup> $P < 0.01$  vs saline-treated group (Student's  $t$ -test); (C) Effect of morphine administration on histamine levels in VTA and NAc from morphine- and saline-treated rats. Data are mean  $\pm$  SEM of 8 observations. <sup>c</sup> $P < 0.01$  vs saline-treated group (Student's  $t$ -test).

**Effect of morphine on and DOPAC/DA ratios in the VTA and NAc** The DOPAC/DA ratio is an index of the activity of dopaminergic neurons<sup>[16]</sup>. We found that morphine administration significantly decreased DA levels and elevated DOPAC/DA ratios in the VTA and the NAc ( $P<0.05$ ; Table 1).

**Effect of histidine on the development of morphine-induced CPP** Morphine-induced CPP depended on the histamine dose ( $F_{3,125}=12.29$ ;  $P<0.01$ ) and number of conditioning sessions ( $F_{2,125}=15.19$ ;  $P<0.01$ ), but there was no interaction between these factors (Figure 4). An analysis of each conditioning session revealed that histidine significantly inhibited the development of morphine-induced CPP at doses of 100 or 200 mg/kg ( $P<0.05$ ) during the first and second conditioning sessions, while during the third conditioning session, only 200 mg/kg histidine was effective ( $P<0.05$ ). Histidine at the high dosage of 200 mg/kg did not induce conditioned place aversion when given alone (data not shown).

**Effect of TM lesions on histamine levels in the VTA and NAc** TM lesions affected histamine levels in the VTA ( $F_{3,32}=12.91$ ;  $P<0.01$ ) and the NAc ( $F_{3,32}=18.30$ ;  $P<0.01$ ; Figure 5). The histamine levels in the VTA and the NAc of the TM lesioned group were markedly decreased relative to the sham

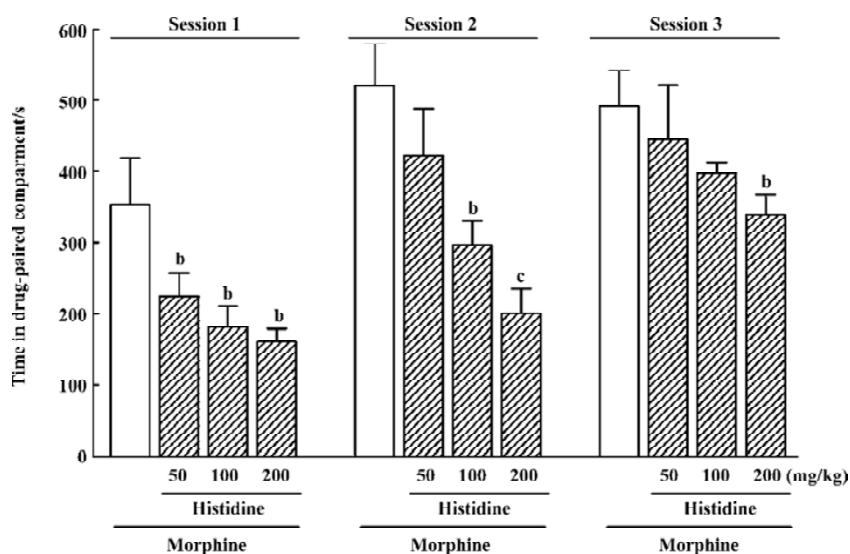
group; however, while the administration of morphine (1 mg/kg) to the TM lesioned group had no further effect on histamine levels in the VTA and NAc, histidine markedly increased histamine levels in these nuclei in the TM lesioned group exposed to morphine.

**Effect of TM lesions on the development of morphine-induced CPP** A two-way ANOVA showed significant effects of the experimental group ( $F_{5,185}=12.76$ ;  $P<0.01$ ), but there was no effect on the number of conditioning sessions or interaction between these variables (Figure 6). Morphine (1mg/kg), TM lesions alone, or sham with morphine had no appreciable effect on the development of CPP, but TM lesions with morphine induced significant CPP relative to sham combined with morphine from the first to third conditioning sessions, while histidine inhibited the CPP induced by TM lesions combined with morphine at the second and third conditioning sessions.

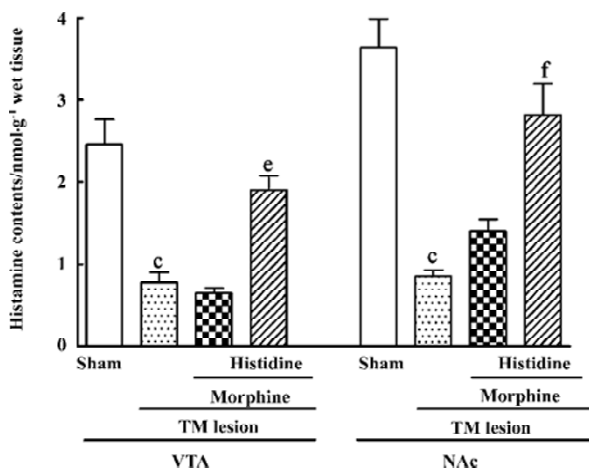
**Effect of TM lesions on glutamate and GABA levels and DOPAC/DA ratios in VTA and NAc** Treatment with morphine, histidine or TM lesions did not change the glutamate or GABA levels in the VTA or the NAc (Table 2). The TM lesion significantly decreased DA and DOPAC lev-

**Table 1.** Effect of morphine on DA, DOPAC and DOPAC/DA ratios in the VTA and NAc. Data are mean±SEM of 8 observations. <sup>b</sup> $P<0.05$ , <sup>c</sup> $P<0.01$  vs saline-treated group (Student's *t*-test).

Groups	VTA			NAc		
	DA (nmol/g)	DOPAC (nmol/g)	DOPAC/DA	DA (nmol/g)	DOPAC (nmol/g)	DOPAC/DA
Saline	22.34±0.09	23.44±1.26	1.08±0.03	20.55±0.86	16.97±1.29	0.84±0.03
Morphine	9.88±0.69 <sup>c</sup>	25.18±1.43	2.49±0.14 <sup>b</sup>	8.39±0.54 <sup>c</sup>	13.48±1.32	1.52±0.56 <sup>b</sup>



**Figure 4.** Effect of histidine on the development of morphine-induced CPP in rats. Morphine (10 mg/kg) was injected ip 5 min before conditioning. Histidine was injected ip 1 h before morphine administration. Data are mean±SEM of time spent in the drug-paired compartment ( $n=10-11$  rats). <sup>b</sup> $P<0.05$ , <sup>c</sup> $P<0.01$  vs saline-treated group during same conditioning session (ANOVA followed by Bonferroni test).



**Figure 5.** Effect of TM lesions on histamine levels in the VTA and NAc. Histidine was administered ip 1 h before morphine administration. Morphine (1 mg/kg) was injected ip 30 min before decapitated. Data are mean±SEM from 8 rats. <sup>c</sup>*P*<0.01 vs sham group, <sup>e</sup>*P*<0.05, <sup>f</sup>*P*<0.01 vs TM lesions combined with morphine (1 mg/kg) group (ANOVA followed by Bonferroni test).

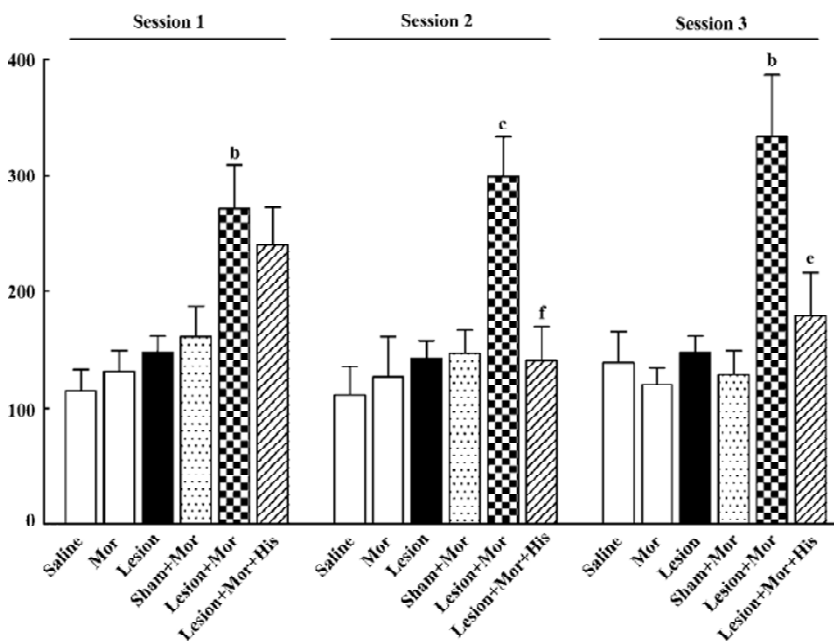
els in the VTA, and decreased DA levels in the NAc (Table 3). After TM lesions, an analysis revealed groups treated with morphine or histidine changed the DOPAC/DA ratios in the VTA ( $F_{3,32}=11.76$ ;  $P<0.01$ ) and NAc ( $F_{3,32}=27.51$ ;  $P<0.01$ ; Table 3). In the VTA, TM lesions changed the DA/DOPAC ratio; lesions combined with morphine increased the ratio, but histidine did not affect this increase. However, in the NAc, the DA/DOPAC ratio significantly increased after TM lesions and lesions combined with morphine markedly

increased the ratio, but histidine significantly reversed this increase.

### Discussion

Morphine is abused for its rewarding and/or euphoric effects, and in animal models, the rewarding effects are demonstrated by the ability to induce CPP<sup>[18]</sup>. In the present study, we found that morphine dose-dependently induced the development of CPP, and 3 administrations of morphine at 10 mg/kg induced significant CPP, as previously shown by others<sup>[19]</sup>. We therefore continued to use this protocol in the present experiments.

The VTA and the NAc are the most likely sites of action for morphine-induced CPP<sup>[20,21]</sup>, so we analyzed the histamine levels in these nuclei after morphine administration. Histamine levels significantly decreased in the VTA and NAc. These decreases may be ascribed to the morphine-induced, long-lasting release of histamine from nerve terminals in the nuclei. Morphine is known to increase the release of histamine from histaminergic neurons<sup>[22,23]</sup>. Since treatment with morphine acutely activates histaminergic neurons intensely and persistently, histamine synthesis may not be sufficient to compensate for the release. Perhaps the decrease in the size and number of histaminergic neurons indicates compensatory adaptations to oppose this intense and persistent histamine release. It is likely that the morphological changes in histaminergic neurons resulted from neural injury caused by exposure to morphine. Although the exact mechanisms remain unclear, our data are the first to indicate



**Figure 6.** Effect of TM lesions on the development of morphine-induced CPP. Morphine (1 mg/kg) was injected ip 5 min before conditioning. Histidine was administered ip 1 h before morphine administration. Data are mean±SEM of time spent in the drug-paired compartment ( $n=10-11$  rats). <sup>b</sup>*P*<0.05, <sup>c</sup>*P*<0.01 vs sham with morphine, <sup>e</sup>*P*<0.05, <sup>f</sup>*P*<0.01 vs TM lesions with morphine (ANOVA followed by Bonferroni test). Mor, morphine (1 mg/kg); Lesion, TM lesions; His, histidine (200 mg/kg).

**Table 2.** Effects of TM lesions on glutamate, GABA levels in the ventral tegmental area and nucleus accumbens. Each value represents the mean±SEM from 8 rats.

Groups	VTA		NAc	
	Glutamate (nmol/g)	GABA (nmol/g)	Glutamate (nmol/g)	GABA (nmol/g)
Sham	211.79±34.45	13.57±2.42	1043.40±153.07	52.20±10.52
TM lesion	309.97±42.53	19.39±2.37	1012.37±135.98	45.26±6.53
TM lesion+Morphine	488.63±18.52	22.05±1.70	1621.06±104.84	53.10±4.14
TM lesion+Morphine+Histidine	427.79±24.43	19.68±0.96	1124.25±59.78	48.74±4.73

**Table 3.** Effects of TM lesions on DA, DOPAC levels and DOPAC/DA ratios in the ventral tegmental area and nucleus accumbens. Each value represents the mean±SEM from 8 rats. <sup>b</sup>*P*<0.05 vs sham, <sup>c</sup>*P*<0.05 vs TM lesion, <sup>d</sup>*P*<0.05 vs TM lesion combined with morphine (1mg/kg) (ANOVA followed by Bonferroni test).

Group	VTA			NAc		
	DA (nmol/g)	DOPAC (nmol/g)	DOPAC/DA	DA (nmol/g)	DOPAC (nmol/g)	DOPAC/DA
Sham	31.27±6.00	34.09±2.84	1.10±0.14	32.25±1.48	19.88±1.42	0.72±0.08
TM lesion	18.08±2.74 <sup>b</sup>	25.84±2.38 <sup>b</sup>	1.68±0.16 <sup>b</sup>	14.97±2.30 <sup>b</sup>	17.64±1.61	1.06±0.05 <sup>b</sup>
TM lesion+morphine 1 mg/kg	11.40±1.16	34.32±2.34	3.14±0.32 <sup>c</sup>	12.64±1.32	23.58±2.06	1.72±0.02 <sup>c</sup>
TM lesion+morphine 1 mg/kg +histidine 200mg/kg	13.65±1.22	34.76±2.28	2.77±0.18	9.69±0.92	9.27±1.44	0.93±0.13 <sup>d</sup>

that endogenous histamine acts as an important neuromodulator in the development of the reward process induced by morphine.

We also found that histidine, a precursor of histamine, produced no conditioned place aversion when given alone, but significantly and dose-dependently inhibited the morphine-induced development of CPP. Histidine at a dose of 200 mg/kg significantly reversed the decreased histamine levels induced by morphine in the VTA and NAc (data not shown). Consistent with this finding, the histidine decarboxylase inhibitor,  $\alpha$ -fluoromethylhistidine, potentiates morphine-induced CPP in mice<sup>[24]</sup>. In addition, it is known that the acquisition and expression of CPP behavior requires learning and memory to establish an association between environmental cues and the effective state induced by morphine; cue reactivity in the CPP paradigm is blocked by scopolamine, which impairs learning and memory<sup>[25,26]</sup>. However, even at the low doses used in the present study, histidine can ameliorate memory deficits in many animal models<sup>[27,28]</sup>. So it is suggested that the inhibitory action of histidine on morphine-induced CPP in the present study is mainly through the environmental cue reactivity associated with the drug.

Furthermore, bilateral electrolytic lesions in the TM, where histaminergic cell bodies are located, reduced histamine lev-

els in the VTA and NAc as expected, and markedly enhanced the development of the reward-seeking behavior in combination with morphine at a dose that had no appreciable effect on CPP in unlesioned animals. In addition, we also found that HDC knockout mice, in which histamine levels in the brain decreases to almost nil in these mice, were much more sensitive to morphine-induced CPP (data not shown). These results strongly support the idea that endogenous histamine participates in the development of the reward-seeking behavior induced by morphine. Previous studies have also shown that TM lesions with DC or ibotenic acid increase the response rates to lateral hypothalamic self-stimulation<sup>[29,30]</sup>, which suggests that the inactivation of TM histaminergic neurons can result in enhanced reinforcement<sup>[31]</sup>. On the other hand, neuroactive substances besides histamine are found in the TM, including GABA, glutamate and adenosine<sup>[32]</sup>. Since electrolytic lesions may non-specifically destroy such neurons or fibers, it was not possible to conclude that the histaminergic neurons were solely responsible for the rewarding effects. However, we found that neither glutamate nor GABA levels in the VTA or NAc changed after TM lesions (Table 2).

We further investigated the effect of histidine as a form of replacement therapy in rats with bilateral TM lesions dur-

ing morphine-induced development of CPP. The precursor reversed the decreasing histamine levels in the VTA and NAc elicited by TM lesions, and facilitated the development of CPP in parallel (Figure 5). Therefore, we concluded that the decreased brain histamine levels, as a consequence of TM lesions, at least in part potentiate the development of morphine-induced reward-seeking behavior. These results provided further evidence that the morphine-induced reward system may be negatively modulated by endogenous histamine.

The DOPAC/DA ratio is used as an index of DA synthesis and metabolism, and represents dopaminergic neuronal activity<sup>[33]</sup>. Brain histamine modulates mesolimbic dopaminergic activity from the VTA to the NAc<sup>[34]</sup>. In this study, we also found that the DOPAC/DA ratios increased in parallel with the decrease of histamine in the VTA and NAc after morphine administration. We found that the TM lesion increased the DOPAC/DA ratio and that histidine reversed this increase. This also suggested that endogenous histamine acts as an inhibitor of the rewarding effect through reducing dopaminergic activity. Decreased brain histamine can weaken the inhibitory effect of dopaminergic activity. Histamine induces hypoactivity and a decreased DOPAC/DA ratio in the striatum<sup>[35]</sup>, while zolantidine, a H<sub>2</sub> receptor antagonist, significantly potentiates morphine-induced CPP by increasing the DOPAC/DA ratio<sup>[24]</sup>. Therefore, DA seems to influence the brain's reward system in a way which is reciprocal to that of histamine; that is, the brain's reinforcement mechanism can be activated by DA moving towards more reinforcement and histamine moving it in the opposite direction<sup>[36]</sup>. An increase in brain histamine levels may attenuate the development of morphine-induced reward-seeking, and its action may be mediated by inhibiting dopaminergic activation in the VTA and NAc. In addition, we found that morphine (1 mg/kg) had no appreciable effect on the DA/DOPAC ratio when given alone, but significantly increased the DA/DOPAC ratio in TM-lesioned rats, which suggests that the activity of the dopaminergic system was facilitated more by morphine when histamine levels were low. So it is proposed that the inhibitory effect on dopaminergic neurons is weakened by a persistent deficiency of histamine, and can further enhance the sensitivity to morphine.

In summary, the present study demonstrates that endogenous histamine is involved in the morphine reward-seeking process and inhibits the development of morphine-induced CPP. The mechanism involves the modulation of dopaminergic activity by endogenous histamine. Therefore, histamine and related compounds may be of value in treating and preventing the development of morphine-induced reward-

seeking behavior.

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