Biphasic modulation of cocaine-induced conditioned place preference through inhibition of histone acetyltransferase and histone deacetylase

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ABSTRACT

الأهداف: دراسة العلاقة المؤثرة بين تغيرات أستيل الهستون الزائغ، و نتيجة الكوكائين الناتجة.

الطريقة: تم اختيار عدد 160 جرذ من نوع سبارغ داولي لآلية تفضيل البيئة المتكيفة (CPP) لتقييم آثار مثبط ديستاليز الهيستون (HDAC)، وناقلة استيل الهستون (HAT) في آثار تكيف الكوكائين. أجريت الدورة المكيفة مرتين يومياً من 2 إلى 4 أيام. في كل دورة مكيفة، تم حقن الجرذان أما بدستاليز الهستون HDAC، أو مثبطات ناقلة الأستيل HAT، أو في المحلول الملحي في القفص، ثم تم حقنهم بالكوكائين أو المحلول الملحي بعد 30 دقيقة، و تقيدهم لمدة 50 دقيقة في غرفة مخصصة. في اليوم التالي، تم اختبار الفئران لاختيار البيئة لمدة 15 دقيقة. أجريت هذه الدراسة في قسم الأدوية – جامعة جكسنج و مركز أبحاث الأدوية – جامعة فودن – شنغهاي – الصين خلال الفترة من أكتوبر 2007م و يناير 2009م.

النتائج: أظهرت نتائج الجرذان من نوع سبارغ داولي والتي تم علاجها مسبقاً بالدستاليز المثبط لبوتيرات الصوديوم HDAC تأيدها للكوكائين الناتج من اختيار البيئة المتكيفة CPP، و لكنه لا يؤدي لاختيارات التكيف أو النفور الشديد. ومن ناحية أخرى، امتنعت جرذان سبارغ داولي التي تم علاجها مسبقاً بناقلة الاستيل المثبط HAT للكركمين من الكوكائين الناتج من اختيار البيئة CPP، ولكنه لم يؤدي لاختيارات التكيف أو النفور.

خاتمة: أشارت هذه البيانات أن تعديل الهيستون يعد آلية مهمة و الذي يظهر آثار تكيف الكوكايين. إضافة إلى ذلك، يعد ناقلة أستيل الهستون HAT عامل علاج ممكن لإدمان الكوكائين.

Objectives: To examine the causative relationship between aberrant histone acetylation changes and cocaine-induced reward.

Methods: Male Sprague-Dawley rats (n=160) were tested by conditioned place preference (CPP) procedure, to evaluate the effects of inhibitors of histone deacetylase (HDAC) and histone acetyltransferase (HAT) on the conditioned effects of cocaine. Conditioning sessions were conducted twice daily for 2-4 days. For each conditioning session, rats were injected with either HDAC (or HAT) inhibitors or saline in home cages, followed by cocaine (intraperitoneally [ip]) or saline (ip) 30 minutes later, and then immediately confined for 50 minutes in the cue-specific chamber. On the day following the last conditioning session, the rats were tested for place preference for 15 minutes. The present study was carried out at the Department of Pharmacology of Jiaxing University, Jiaxing, Zhejiang, and Pharmacology Research Center of Fudan University, Shanghai, China between October 2007 and January 2009.

Results: Our results showed that pretreatment with HDAC inhibitor (sodium butyrate), potentiated cocaine-induced CPP, but did not itself lead to conditioned preferences, or aversions. On the contrary, rats pretreated with curcumin (HAT inhibitor) markedly inhibited cocaine-induced CPP, but did not itself lead to conditioned preferences or aversions.

Conclusion: Histone modifications may be an important mechanism that underlies conditioned effects of cocaine. Moreover, HAT may be a potential therapeutic target for cocaine addiction.

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Drug addiction is currently viewed as a chronic brain disease characterized by a compulsive drug-seeking and drug-taking behavior.[1] The complex behavioral alterations leading to drug addiction require the molecular basis of stable changes in specific brain regions.[2] Regulation of gene expression is considered a plausible mechanism of the neural and behavioral plasticity associated with cocaine addiction. For instance, cocaine exposure during adolescence increased expression of genes encoding cell adhesion molecules, and transcription factors within the prefrontal cortex.[3] Acute cocaine administration upregulated transcripts included immediate-early genes, scaffolding proteins, and signal transduction protein.[4-6] Chronic cocaine administration elicited induction in neurons of several transcripts such as protein kinase A alpha, metabotropic glutamate receptor 5, and voltage-gated potassium channel 1.1, survival of motor neuron, and protein phosphatase 2A alpha subunit in the hippocampus.[7] Increasing evidence indicates that histone modifications, particularly histone acetylation, are important regulatory mechanisms for cocaine-induced gene expression. Acute cocaine elicits the expression of cFos in the striatum, and this induction is associated with H4 hyperacetylation at cFos promoter.[8] Chronic cocaine increased H3 hyperacetylation at the brain-derived neurotrophic factor and cyclin-dependent kinase 5 promoters.[8] In addition, loss of histone deacetylase (HDAC) 5 increased histone acetylation at specific genes, and ultimately enhanced reward behavior to chronic cocaine.[9] The data suggest that the histone acetylation regulation mechanism of gene transcription could be involved in cocaine addiction. Histone acetylation is a dynamic process, controlled by enzymes that either add or remove acetyl groups onto lysine residues of histone proteins. In general, increased histone acetylation by histone acetyltransferases (HATs) is associated with DNA relaxation and elevated transcriptional activity conversely decreased acetylation brought on by HDACs, results in tighter DNA coiling, and gene repression.[10,11] Sodium butyrate (NaBu), a known HDAC inhibitor, has been demonstrated to cause an increasing histone acetylation via inhibiting HDAC enzyme *in vitro* and *in vivo*.[12] A recent study showed that NaBu induces histone H3 phosphoacetylation in striatum when administrated prior to cocaine.[8] Curcumin, a novel p300/cyclic adenosine monophosphate (cAMP) response element-binding (CREB-binding) protein-specific inhibitor of HATs, decreases histone acetylation.[13] Until now, the causative relationship between aberrant histone acetylation changes and cocaine-induced reward remains mainly unclear. To that effect, we investigated the effects of NaBu, a HDACs inhibitor, and curcumin, an inhibitor of HATs, on cocaine induced addictive behavior using a conditioned place preference (CPP) model that has been used for the study of addiction.

Methods. ***Animals, reagents, and apparatus.*** Male Sprague-Dawley rats (n=160), 220-250 g, and 12 weeks old (Shanghai Center of Experimental Animal, and Chinese Academy of Sciences, Shanghai, China), were included in this study. The rats were housed in a temperature-controlled animal facility, and maintained on a 12 hour light/dark cycle (lights on from 18:30-06:30 hours). Animals had *ad libitum* access to food and water. The present study was carried out at the Department of Pharmacology of Jiaxing University and Pharmacology Research Center of Fudan University, China between October 2007 and January 2009. The experiments were performed according to the national regulations and approved by the local animal experiments ethical committee.

Cocaine hydrochloride was purchased from Qinghai Pharmaceutical Firm, Qinghai, China, and dissolved in physiological saline. The NaBu (Sigma Chemical Co., St. Louis, MO, USA) was dissolved in saline. Curcumin was obtained from Sinopharm Chemical Reagent Co. Ltd., Shanghai, China. Curcumin was dissolved in 5 N sodium hydroxide, and titrated to pH 7.4 using one N hydrogen chloride, and was diluted with physiological saline. All the solutions were freshly prepared for the experiments. The CPP apparatus consisted of a shuttle chamber made of plexiglass (30 cm x 60 cm x 30 cm), and divided into 2 equal-sized compartments by the insertion of a removable plexiglass wall. The device was black, one compartment had white strips on the wall and a textured floor, and the other had walls with white dots and a smooth floor. The apparatus was placed in a sound attenuation cubicle.

CPP procedure. The CPP was conducted using an unbiased procedure according to the method of Wang et al.[14] On the day before the experiment began, the removable plexiglass wall separating the 2 compartments was raised above the floor, rats were placed on the floor of the test box, and allowed to move freely to habituate the test box for 15 minutes. On day one, the rats were also allowed to move freely in the test box for 15 minutes. The amount of time spent in each compartment was recorded to assess unconditioned preference. The rats were then randomly divided into 4 groups (n=10) for each subsequent experiment. Conditioning sessions were conducted twice daily for 2-4 days depending on different experiments. Cocaine was always paired with a cue-specific chamber for 50 minutes, and saline was paired with the other chamber for 50 minutes; cocaine or saline exposure (and appropriate environmental pairing) were alternated at 08:00 and 16:00 hours. For each conditioning session, rats were injected with either HDAC (or HAT) inhibitors or saline in the home cage,

followed 30 minutes later by cocaine (intraperitoneally [ip]) or saline (ip), and then immediately confined for 50 minutes in the cue-specific chamber. On the day following the last conditioning session, the rats were tested for place preference for 15 minutes.

First experiment. Effect of NaBu on the acquisition of CPP induced by cocaine. In the experiment, the conditioning sessions were conducted for 2 days as described previously by McGeehan et al.[15] In each conditioning session, animals were injected with NaBu (100 or 200 mg/kg, ip) or the same volume of saline, just before cocaine (2 mg/kg, ip) administration. On the day following the last conditioning session, the rats were tested for CPP for 15 minutes. As a control for possible state-dependent learning influences of NaBu, rats received daily NaBu (100, 200 mg/kg, ip), cocaine (2 mg/kg, ip) or saline in equal volumes for 2 days. On the day following the last conditioning session, the rats were tested for CPP for 15 minutes. The time spent in each compartment during a 15-minute session was recorded, and preference to a drug-paired compartment was determined as time spent in the drug-paired side in the session.

Second experiment. Effect of curcumin on the acquisition of CPP induced by cocaine. To test the effects of curcumin on the acquisition of CPP induced by cocaine, the conditioning sessions were conducted twice daily for 4 days. In each conditioning session, rats were injected with either curcumin (10, or 30 mg/kg, ip) or the same volume of saline in the home cage, followed by cocaine (10 mg/kg, ip) or saline (ip) 30 minutes later, and then immediately confined for 50 minutes in the cue-specific chamber. On the day following the last conditioning session, the rats were tested for CPP for 15 minutes. As a control for possible state-dependent learning influences of curcumin, rats received daily curcumin (10, or 30 mg/kg, ip), cocaine (10 mg/kg, ip) or saline for 4 days. On the day following the last conditioning session, the rats were tested for CPP for 15 minutes.

Data for CPP were analyzed by one-way analysis of variance, with a posteriori individual group comparisons by the Tukey test for multiple comparisons, when appropriate. In all statistical tests, a value of $p<0.05$ was considered to be significant. Statistical analyses were conducted using the Statistical Package for Social Sciences for Windows version 11 (SPSS Inc., Chicago, Illinois, USA).

Results. The NaBu enhanced the acquisition of cocaine-CPP. As shown in **Figure 1a**, cocaine training (2 mg/kg, ip) for 2 consecutive days significantly increased the time spent in the drug-paired chamber as compared with the saline group ($p=0.017$), while NaBu had no significant effect (NaBu 100mg/kg versus saline, $p=0.66$; NaBu 200mg/kg versus saline, $p=0.75$). These results indicate that NaBu itself could not induce place preference or place aversion. **Figure 1b** shows that pretreatment with NaBu at 100 or 200 mg/kg, 30 minutes before daily cocaine training increased the time spent in the drug-paired chamber (NaBu 100 mg/kg + cocaine group versus cocaine group, $p=0.013$; NaBu 200 mg/kg + cocaine group versus cocaine group, $p=0.011$). These data reveal that NaBu, enhances acquisition of cocaine-induced CPP.

Curcumin decreased the acquisition of cocaine-CPP. As shown in **Figure 2a** cocaine training (10 mg/kg, ip) for 4 consecutive days significantly increased the time spent in the drug-paired chamber as compared with the saline group ($p=0.019$), while curcumin had no significant effect (curcumin 10 mg/kg versus saline, $p=0.29$; curcumin 30 mg/kg versus saline, $p=0.10$). These results indicate that curcumin itself could not

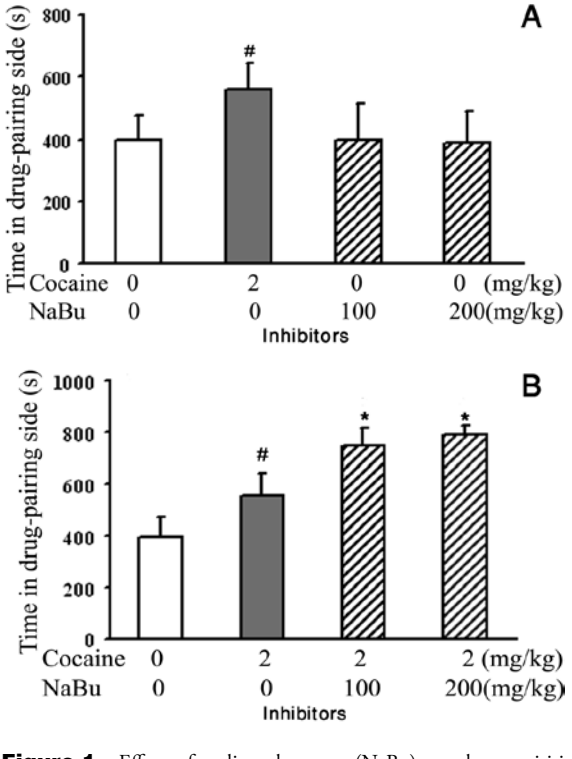

Figure 1 - Effect of sodium butyrate (NaBu) on the acquisition of cocaine induced place preference, **a**) NaBu (100, or 200 mg/kg intraperitoneally [ip]) itself does not produce conditioned place preference. **b**) NaBu enhances the acquisition of cocaine-induced place preference. The bars represent the mean ± standard error of the mean (SEM) time spent in the drug paired side (n=10 per group). Data were expressed as the mean ± SEM. Cocaine (2 mg/kg) group versus saline control, $^{\#}p<0.05$; NaBu 100 mg/kg + cocaine group versus cocaine group, and NaBu 200 mg/kg + cocaine group versus cocaine group, $^{*}p<0.05$; one-way analysis of variance followed by Tukey test. Shading indicates the time in drug-pairing side of rats in the control group.

induce place preference or place aversion. **Figure 2b** shows the effect of different doses of curcumin on place preference. Analysis showed a significant effect for curcumin on place preference. Further analysis revealed that pretreatment with curcumin at 30 mg/kg before daily cocaine training decreased the time spent in the drug-paired chamber (p=0.006 compared with the cocaine group); however, curcumin at 10 mg/kg failed to produce a significant effect (p=0.16 compared with the cocaine group). These data substantiate that curcumin, an inhibitor of HATs, antagonizes acquisition of cocaine-induced CPP.

Discussion. Our results suggest that cocaine-induced CPP can be biphasically modulated by inhibition of HATs and HDACs activity. These 2 groups of enzymes, HDACs and HATs, determine the pattern of histone acetylation. Histone acetylation/deacetylation mechanisms control modifications of chromatin structure that results in the regulation of gene transcription. Of all the known chromatin modifications, histone acetylation has the most potential to unfold chromatin, and has received much attention in the nervous system.[16,17] Several recent studies have identified the regulation of histone acetylation as an important regulator of stimulant-related plasticity and addiction behavior.[8,9,18]

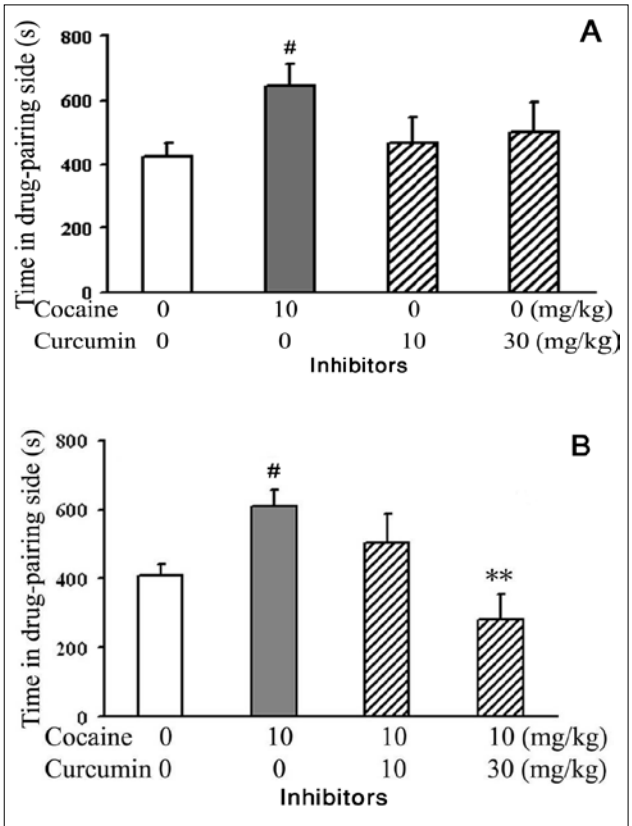

Figure 2 - Effect of curcumin on the acquisition of cocaine-induced place preference **a**) curcumin (10, or 30 mg/kg intraperitoneally [ip]) itself does not produce conditioned place preference. **b**) curcumin decreases the acquisition of cocaine-induced place preference. The bars represent the mean ± standard error of the mean (SEM) time spent in the drug paired side (n=10 per group). Cocaine 10 mg/kg group versus saline control, $^{\#}p<0.05$; curcumin 30 mg/kg + cocaine group versus cocaine group, $^{**}p<0.05$; by one way analysis of variance followed by Tukey test. The columns represent time spent in the drug paired side (n=10 per group). Data were expressed as the mean ± SEM of the time. The shading indicates the time in drug-pairing side of rats in the control group.

Previous studies showed that NaBu, increased acetylation of histones in intact cells, and is being explored as treatments for several neurodegenerative diseases, such as Huntington's disease and depression.[19-21] The NaBu enhanced the induction of long-term potentiation in hippocampal slices using high-frequency stimulation.[22] Administration of NaBu followed by cocaine caused a dramatic increase in the level of histone H3 phosphoacetylation in the striatum.[8] The NaBu at a dose 400 mg/kg increases cocaine-induced lever presses during the maintenance phase in cocaine self-administration paradigm.[23] Notably, a recent study[24] showed that cotreatment with D1 agonist and NaBu significantly enhanced cocaine-induced locomotor activity and place preference in mice; furthermore, the effects of NaBu were transient and only apparent within 2 days after the last treatment of NaBu. In this study, we found NaBu significantly enhanced the acquisition of cocaine-induced CPP in rats. Our results are in agreement with a study showing that treatment of mice with another HDAC inhibitor, trichostatin A, potentiates cocaine-induced place conditioning.[8] It is also consistent with the findings that mice receiving intra-nucleus accumbens delivery of suberoylanilide hydroxamic acid, a highly specific inhibitor HDACs, augments rewarding responses to cocaine.[21] The mechanism underlying the effect of NaBu on cocaine-induced CPP is unknown. One possible explanation is that histone hyperacetylation, after the inhibition of HDACs, elicits specific gene expression and consequently enhance cocaine-induced CPP. These findings further substantiate the concept that the rewarding effects of cocaine can be enhanced through the inhibition of HDAC.

It has been shown that histone acetylation can be increased by HATs. Recently, Levine et al[18] showed that histone acetylation by the cAMP-response element binding (CREB) protein, a HAT, mediates sensitivity to cocaine by regulating expression of the fosB gene. Our results showed that in rats treated with NaBu, cocaine-induced reward behavior is enhanced. It is therefore tempting to speculate that the opposite strategy, that is treatment with a HAT inhibitor could perhaps prevent, or attenuate, some of the behaviors associated with cocaine

reward. Curcumin induces histone hypoacetylation in brain cancer cells. In addition, curcumin induces effective neurogenesis, synaptogenesis, and migration of neural progenitor cells *in vitro* in brain-derived adult neural stem cells.[25] To date, the effects of curcumin on the conditioned effect of cocaine remains unclear. Our results showed that curcumin, a molecule that decreases histone acetylation by inhibiting HATs, attenuates acquisition of CPP produced by cocaine. The decreased behavioral responses to cocaine are likely to result from histone hypoacetylation after the inhibition of HATs to silence specific gene expression, and consequently alter neuronal function.

Our study demonstrates that modulation of HDAC and HAT activity can alter cocaine-induced CPP. Histone acetylation/deacetylation may play a key role in this process. Our findings suggest that the proper balance of histone acetylation is a crucial factor in the salience of cocaine stimulus, and HAT inhibitors may promise as a potential therapeutic agents for cocaine addiction, but further research is required for confirmation.

Although the results were encouraging, it must be taken into consideration, that the evaluation of the effects of HDAC or HAT inhibitors on cocaine addiction was limited by CPP procedure, and only to NaBu and curcumin. Therefore, further study is needed, tested by other addiction model such as, cocaine self-administration procedures, and with other inhibitors of HDAC or HAT to confirm its effectiveness and molecular mechanisms.

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