

Comparison of the sensitizing effects of cocaine and ethanol on histone deacetylase isoforms in the rat brain

Shijie Xu^{a,b,c}, Ja Wook Koo^{d,e,*} and Ung Gu Kang^{b,f,*}

Behavioral sensitization, an animal model of drug addiction, persists for a prolonged period after repeated exposure to drugs of abuse. The persistence of an addiction behavioral phenotype suggests long-lasting changes in gene regulation at the epigenetic level. We measured the expression of histone deacetylases (HDACs) isoforms in the prefrontal cortex and dorsal striatum following the development of sensitization to cocaine (15 mg/kg, administered five times) and ethanol (0.5 g/kg, administered 15 times) to investigate the epigenetic changes that mediate sensitization. Animals sensitized to ethanol exhibited augmented locomotor activity in response to the cocaine challenge. Similarly, those sensitized to cocaine exhibited increased locomotor activity in response to an ethanol challenge. These findings indicate cross-sensitization between ethanol and cocaine and suggest that a common molecular mechanism underlying the cross-sensitization. In animals sensitized to cocaine or ethanol, mRNA levels of class II HDACs (HDAC4 and HDAC5) were decreased in the prefrontal cortex and dorsal striatum, whereas acute treatments with either drug had no effect on the expression of class II HDACs. By contrast, class I HDACs (HDAC1 and HDAC2) responded to the acute

cocaine challenge, whereas sensitization itself did not have a consistent effect on class I HDAC levels. These findings support the hypothesis of a common epigenetic mechanism underlying persistent behavioral sensitization induced by different drugs, which may be mediated by the altered expression of class II HDACs. *NeuroReport* 32: 423–430 Copyright © 2021 Wolters Kluwer Health, Inc. All rights reserved.

NeuroReport 2021, 32:423–430

Keywords: behavioral sensitization, cocaine, ethanol, histone deacetylases

^aMedical Research Center, Hainan Cancer Hospital, Haikou, Hainan, China, ^bInstitute of Human Behavioral Medicine, Medical Research Center, Seoul National University, ^cBiomedical Research Institute, Seoul National University Hospital, Seoul, ^dEmotion, Cognitive & Behavior Research Group, Korea Brain Research Institute, 61, Cheomdan-ro, Dong-gu, ^eDepartment of Brain and Cognitive Sciences, Daegu Gyeongbuk Institute of Science and Technology (DGIST), Daegu and ^fDepartment of Psychiatry and Behavioral Science, Seoul National University College of Medicine, Seoul, Republic of Korea

Correspondence to Ung Gu Kang, MD, Department of Psychiatry and Behavioral Science, Seoul National University College of Medicine, Seoul 03080, Republic of Korea

Tel: +82 2 2072 2296; fax: +82 2 744 7241; e-mail: kangug@snu.ac.kr

*These authors contributed equally to the writing of this article.

Received 4 October 2020 Accepted 20 November 2020

Introduction

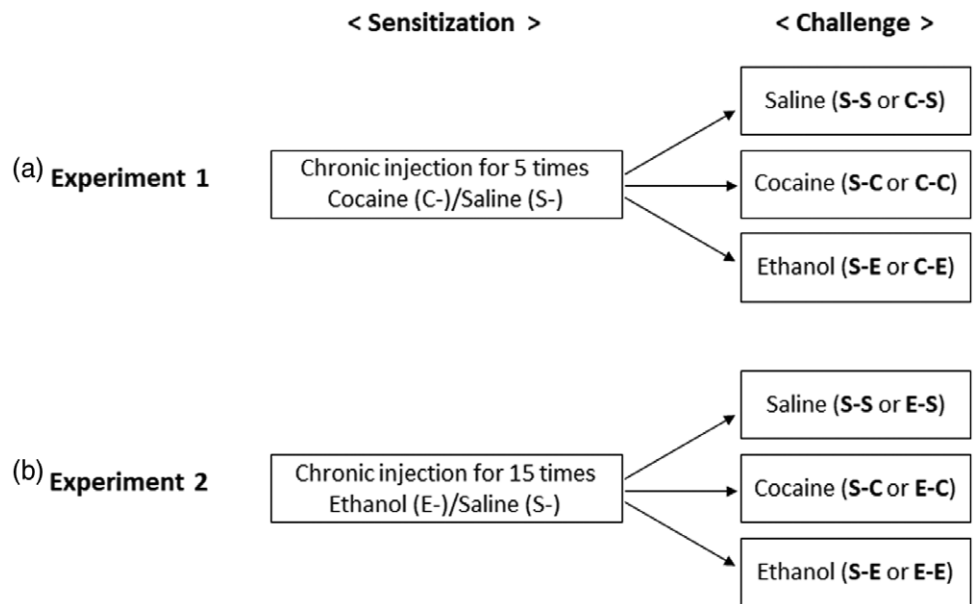
Repeated exposure to drugs of abuse enhances behavioral responses; a phenomenon referred to as behavioral sensitization [1–3]. In the behavioral sensitization paradigm, the sensitized state induced by chronic drug use is long-lasting [4–6], and sensitization to one drug may influence susceptibility to other drugs [7–11]. Previously, we reported cross-sensitization between cocaine and ethanol, suggesting the presence of shared common neural and cellular mechanisms related to behavioral sensitization [12,13]. Epigenetic modification may be a key cellular process underlying drug-induced persistent behavioral changes [14–17]. However, it is not clear how the sensitized state is related to epigenetic changes.

A common epigenetic modification site is the N-terminal lysine residues, which can be reversibly acetylated by histone acetyltransferases or deacetylated by several histone deacetylases (HDACs) [15,18,19]. Deacetylated histones promote the condensation of chromatin, leading to a reduction in transcription activity [20,21]. HDACs are divided into four classes: class I (HDAC1, HDAC2,

HDAC3 and HDAC8), class II (HDAC4, HDAC5, HDAC6, HDAC7, HDAC9 and HDAC10), class III (sirtuins 1–7) and class IV (HDAC11) [17,22,23]. Previous studies have focused on changes in the activity and expression of class I and II HDACs associated with long-term drug treatment. In these studies, long-lasting behavioral changes were considered to indicate involvement in the maintenance of addiction-related behaviors [22–25]. However, the behavioral effects of various addictive drugs are not consistent, and contradictory findings have been reported for cocaine, amphetamine, morphine and alcohol [26–29].

To determine whether drugs that induce behavioral sensitization share common epigenetic mechanisms, we studied the expressions of class I (HDAC1 and HDAC2) and II (HDAC4 and HDAC5) HDACs in the prefrontal cortex and dorsal striatum following the development of behavioral sensitization induced by cocaine or ethanol. Previous studies have shown that persistent sensitization induces long-lasting changes in the mesocortico-striatal dopaminergic system [30–32]. Moreover, cocaine- or ethanol-induced histone modifications in the prefrontal

Fig. 1



Experimental schedule. Behavioral sensitization was induced by chronic injections of cocaine (C; 5 times, 15 mg/kg, experiment 1) or ethanol (E; 15 times, 0.5 g/kg, experiment 2). After sensitization was established, the animals were challenged with saline (S-S, C-S or E-S), cocaine (S-C, C-C or E-C), or ethanol (S-E, C-E or E-E).

Table 1 Primer for qRT-PCR

	Forward (5'–3')	Reverse (5'–3')
HDAC1	AGTATAATGTCCCCAGGGACCA	CTGAAAAGGGGCCCCAGAAG
HDAC2	CGGCAAGAAGAAAGTGTGCT	TCCATCGAACACTGGACAGT
HDAC4	TGAGAGACGGAGCAGCCCCC	GGCGCTGCTACATGCGGAGT
HDAC5	TTCTTCAACTCCGTAGCC	TCCCATTGTCGTAGCG
β-actin	CCTCTGAACCCTAAGGCCAA	AGCCTGGATGGCTACGTACA

qRT-PCR, quantitative real-time PCR.

cortex and dorsal striatum could play a crucial role in the transition to addiction-like behavior [17,33,34].

Methods

Animals and drugs

Male Sprague–Dawley rats (150–200 g; KoaTech, Gyeonssi-do, Korea) were maintained under a 12/12 h light/dark cycle (lights on at 06:00) with water and food available ad libitum. Cocaine hydrochloride (15 mg/kg, Belgopia; Louvain-La-Neuve, Belgium) and ethanol (0.5 g/kg, Merck; Whitehouse Station, New Jersey, USA; cat. #1.00983.1011) were diluted in 0.9% saline and administered intraperitoneally. This study was approved by the Institutional Animal Care and Use Committee at Seoul National University Hospital (SNUH-IACUC) and the animals were maintained in a facility accredited by the Association for Assessment and Accreditation or Laboratory Animal Care International (#001169) in accordance with the Guide for the Care and Use of Laboratory Animals 8th edition, National Research Council [35].

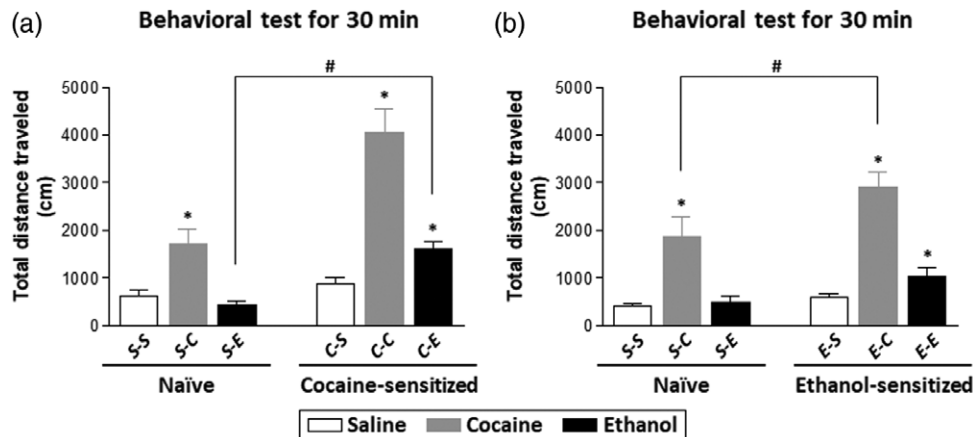
Drugs treatment schedule

Two sets of experiments were performed (Fig. 1). For experiment 1, rats were randomly assigned to receive saline (S) or cocaine (C) daily for 5 days; these chronic treatments were designated as 'sensitization'. After the fifth treatment, the rats were further assigned to one of three subgroups ($n = 6$ or 7 per subgroup) in which they received one injection of saline (subgroups S-S and C-S), cocaine (subgroups S-C and C-C) or ethanol (subgroups S-E and C-E); these treatments were designated as 'challenge'. Following the challenge, locomotor activity was recorded for 30 min to assess behavioral sensitization. For experiment 2, rats were randomly assigned to receive saline (S-) or ethanol (E-) five times per week (Monday to Friday between 10:00 and 16:00) for 3 weeks (15 injections). After the 15th treatment, the rats in each group were assigned to one of three subgroups ($n = 6$ or 7 per subgroup) and challenged with saline (subgroups S-S and E-S), cocaine (subgroups S-C and E-C), or ethanol (subgroups S-E and E-E). Then locomotor activity was recorded for 30 min. Following the measurement of locomotor activity, the rats were immediately decapitated, and the prefrontal cortex and the dorsal striatum were obtained. The treatment regimen for sensitization to cocaine, ethanol and reciprocal cross-sensitization were performed according to a previous study [13].

Measurement of locomotor activity

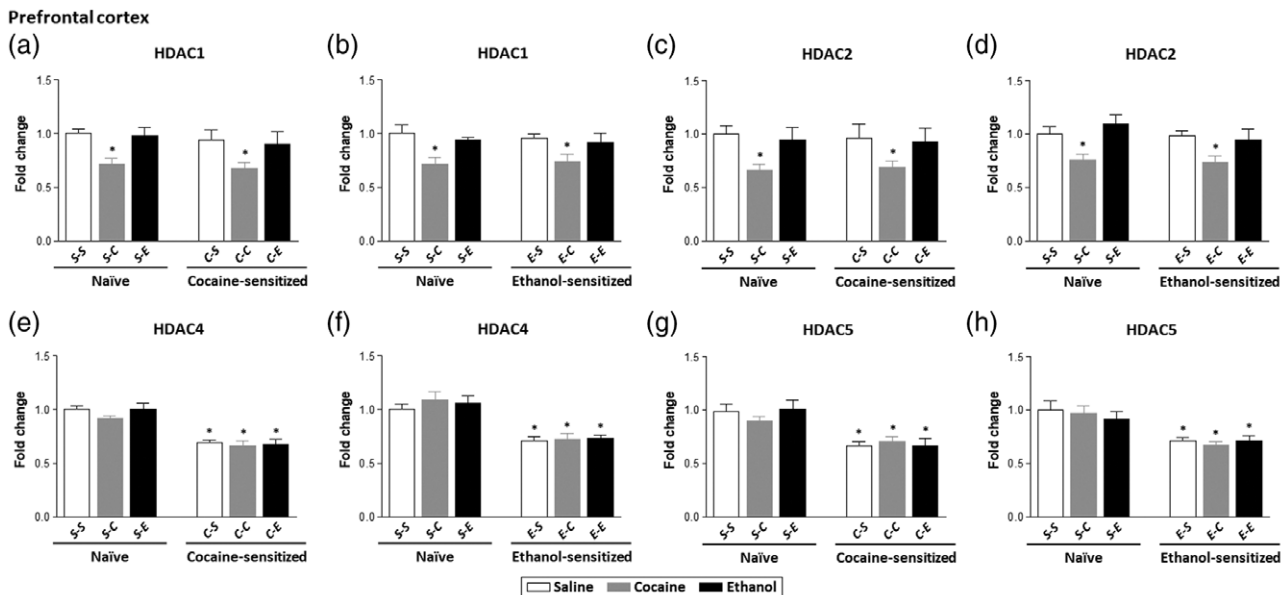
Locomotor activity was recorded in a transparent acrylic box (42 × 42 × 30 cm) using a video-tracking

Fig. 2



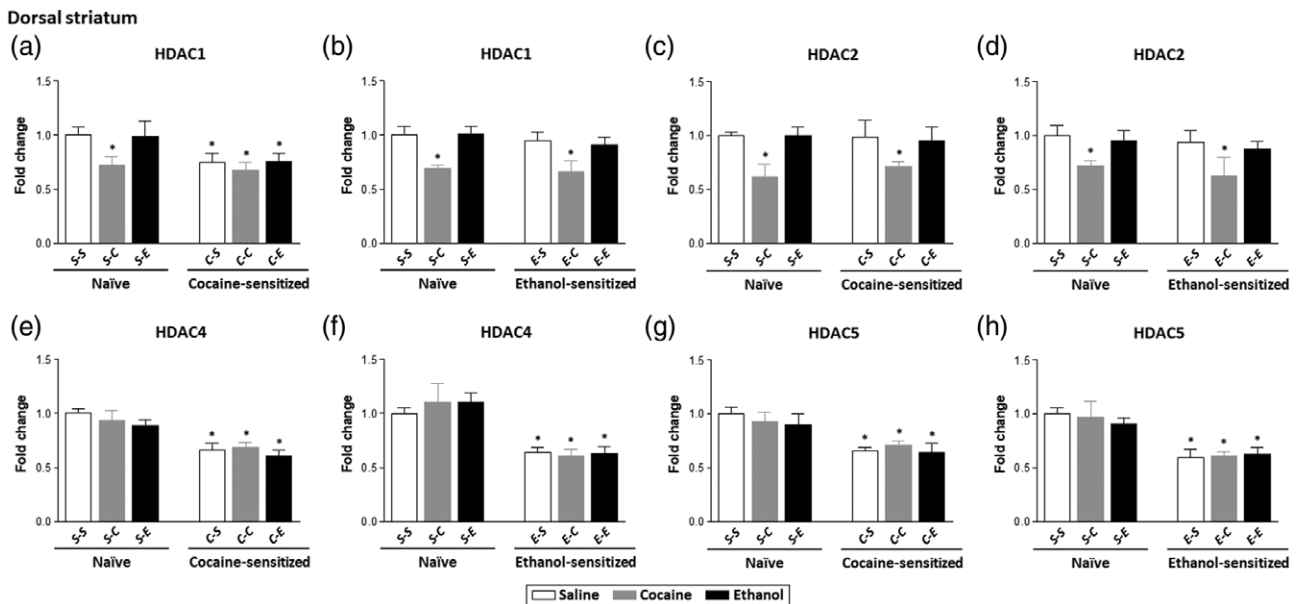
Behavioral cross-sensitization between cocaine and ethanol. Effects of saline (0.9% NaCl, -S), cocaine (15 mg/kg, -C) or ethanol (0.5 g/kg, -E) challenges to animals sensitized to cocaine (5 injections, C-, panel A) or ethanol (15 injections, E-, panel B). Locomotor activity was recorded for 30 min. All data are expressed as means $\pm$ SE ($N = 6$ or 7 rats/subgroup). * $P < 0.05$, vs. S-S naïve subgroup, # $P < 0.05$, significant differences between S-C vs. E-C and S-E vs. C-E, two-way ANOVA followed by Tukey post hoc tests.

Fig. 3



mRNA levels of HDAC1, HDAC2, HDAC4 and HDAC5 in the prefrontal cortex following reciprocal cross-sensitization between cocaine (15 mg/kg) and ethanol (0.5 g/kg). Fold changes in the expression of each gene compared to the control group were normalized to β -actin expression (mRNA). Data are expressed as means $\pm$ SE ($N = 6$ or 7 rats/group). (a) HDAC1 (two-way ANOVA with Tukey's post hoc test, challenge effect: $F(2, 42) = 7.462$, $P = 0.003$; $P = 0.003$, S-S vs. S-C; $P = 0.001$, S-S vs. C-C; $P = 0.842$, S-S vs. S-E; $P = 0.438$, S-S vs. C-E; $P = 0.558$, S-S vs. C-S). (b) HDAC1 (two-way ANOVA with Tukey's post hoc test, challenge effect: $F(2, 42) = 8.149$, $P = 0.002$; $P = 0.049$, S-S vs. E-C; $P = 0.546$, S-S vs. E-E; $P = 0.650$, S-S vs. E-S). (c) HDAC2 (two-way ANOVA with Tukey's post hoc test, challenge effect: $F(2, 42) = 5.551$, $P = 0.010$; $P = 0.009$, S-S vs. S-C; $P = 0.016$, S-S vs. C-C; $P = 0.720$, S-S vs. S-E; $P = 0.652$, S-S vs. C-E; $P = 0.801$, S-S vs. C-S). (d) HDAC2 (two-way ANOVA with Tukey's post hoc test, challenge effect: $F(2, 42) = 8.356$, $P = 0.002$; $P = 0.021$, S-S vs. E-C; $P = 0.664$, S-S vs. E-E; $P = 0.867$, S-S vs. E-S). (e) HDAC4 (two-way ANOVA with Tukey's post hoc test, sensitizing effect: $F(2, 42) = 59.083$, $P < 0.001$; $P = 0.006$, S-S vs. E-C; $P = 0.003$, S-S vs. E-E; $P = 0.001$, S-S vs. E-S). (f) HDAC4 (two-way ANOVA with Tukey's post hoc test, sensitizing effect: $F(2, 42) = 33.143$, $P < 0.001$; $P = 0.008$, S-S vs. C-C; $P = 0.010$, S-S vs. C-E; $P = 0.003$, S-S vs. C-S). (g) HDAC5 (two-way ANOVA with Tukey's post hoc test, sensitizing effect: $F(2, 42) = 29.936$, $P < 0.001$; $P = 0.006$, S-S vs. E-C; $P = 0.018$, S-S vs. E-E; $P = 0.008$, S-S vs. E-S). * $P < 0.05$, vs. S-S control subgroup.

Fig. 4



mRNA levels of HDAC1, HDAC2, HDAC4 and HDAC5 in the dorsal striatum following reciprocal cross-sensitization between cocaine (15 mg/kg) and ethanol (0.5 g/kg). Fold changes in gene expression compared to the control group were normalized to β -actin expression (mRNA). Data are expressed as means $\pm$ SE ($N = 6$ or 7 rats/group). (a) HDAC1 (two-way ANOVA with Tukey's post hoc test, sensitizing effect: $F(2, 42) = 5.648$, $P = 0.025$; $P = 0.021$, S-S vs. S-C; $P = 0.513$, C-S vs. C-C; $P = 0.048$, S-S vs. C-S; $P = 0.954$, C-S vs. C-E). (b) HDAC1 (two-way ANOVA with Tukey's post hoc test, challenge effect: $F(2, 42) = 8.003$, $P = 0.002$; $P = 0.032$, E-S vs. E-C; $P = 0.915$, S-S vs. S-E; $P = 0.650$, S-S vs. E-E). (c) HDAC2 (two-way ANOVA with Tukey's post hoc test, challenge effect: $F(2, 42) = 6.595$, $P = 0.005$; $P = 0.004$, S-S vs. S-C; $P = 0.040$, C-S vs. C-C; $P = 0.903$, S-S vs. C-S). (d) HDAC2 (two-way ANOVA with Tukey's post hoc test, challenge effect: $F(2, 42) = 4.046$, $P = 0.031$; $P = 0.031$, E-S vs. E-C; $P = 0.682$, S-S vs. E-S; $P = 0.769$, S-S vs. S-E; $P = 0.379$, S-S vs. E-E). (e) HDAC4 (two-way ANOVA with Tukey's post hoc test, sensitizing effect: $F(2, 42) = 35.507$, $P < 0.001$; $P = 0.748$, C-S vs. C-C; $P = 0.551$, C-S vs. C-E; $P = 0.002$, S-S vs. C-S). (f) HDAC4 (two-way ANOVA with Tukey's post hoc test, sensitizing effect: $F(2, 42) = 48.976$, $P < 0.001$; $P = 0.756$, E-S vs. E-C; $P = 0.980$, E-S vs. E-E; $P < 0.001$, S-S vs. E-S). (g) HDAC5 (two-way ANOVA with Tukey's post hoc test, sensitizing effect: $F(2, 42) = 19.015$, $P < 0.001$; $P = 0.373$, C-S vs. C-C; $P = 0.890$, C-S vs. C-E; $P = 0.002$, S-S vs. C-S). (h) HDAC5 (two-way ANOVA with Tukey's post hoc test, sensitizing effect: $F(2, 42) = 24.245$, $P < 0.001$; $P = 0.877$, E-S vs. E-C; $P = 0.776$, E-S vs. E-E; $P = 0.002$, S-S vs. E-S). * $P < 0.05$, vs. S-S control subgroup.

apparatus (Activity Monitor 5.0; Med Associates, St. Albans, Vermont, USA) located in a sound-attenuated test cubicle. Each rat was placed in the box and allowed to habituate for 30 min. Then the animal was challenged with saline or the designated drug, and locomotor activity was recorded in 5 min bins for an additional 30 min period.

Quantitative real-time PCR

Total RNA was extracted from frozen tissues using TRI reagent (Molecular Research Center, Cincinnati, Ohio, USA), as previously described [12]. Then, 1 μ g total RNA was reverse-transcribed to cDNA using the Superscript II RT system (Invitrogen, Carlsbad, California, USA). Quantitative real-time PCR was performed using an ABI PRISM 7000 instrument (Applied Biosystems, Foster City, California, USA) with the SYBR Green real-time PCR master mix (Toyobo, Osaka, Japan) according to the manufacturer's instructions. The reaction was first incubated at 50°C for 2 min, then at 95°C for 10 min, followed by 35 cycles at 95°C for 15 s and 60°C for 1 min. All assays were performed in triplicate, and relative

amounts of all mRNAs were calculated using the comparative Ct method using the $2^{-\Delta\Delta C_t}$ equation, as previously described [36]. The primer sequences for quantitative real-time PCR are shown in Table 1.

Statistical analysis

Data were analyzed using two-way analyses variance (ANOVA) tests followed by post hoc Tukey tests. All tests were performed using the version 22.0 SPSS software package for Windows (SPSS Inc., Chicago, Illinois, USA). A P value < 0.05 was considered to indicate statistical significance.

Results

Behavioral cross-sensitization between cocaine and ethanol

Locomotor activity significantly increased following repeated treatment and acute challenge with cocaine and ethanol [interaction between sensitization and challenge: $F(2, 42) = 6.164$, $P = 0.005$, Fig. 2a; interaction between sensitization and challenge: $F(2, 42) = 3.535$, $P = 0.038$, Fig. 2b]. Post hoc tests revealed that a single injection of

cocaine increased locomotor activity ($P = 0.001$, S-S vs. S-C, Fig. 2a) in naïve animals, whereas ethanol had no effect on locomotor activity ($P = 0.290$, S-S vs. S-E, Fig. 2a). The locomotor activity of the naïve group in experiment 2 was similar to that in experiment 1 ($P < 0.001$, S-S vs. S-C; $P = 0.516$, S-S vs. S-E, Fig. 2b, respectively). Neither cocaine (C-S) nor ethanol (E-S) sensitization per se affected locomotor activity compared to the naïve (S-S) group ($P = 0.169$, S-S vs. C-S, Fig. 2a; $P = 0.116$, S-S vs. E-S, Fig. 2b). However, the behavioral responses to the cocaine and ethanol challenges were greater in sensitized animals than in naïve animals challenged with the same drugs ($P < 0.001$, S-E vs. C-E, Fig. 2a; $P = 0.036$, S-C vs. E-C, Fig. 2b), thus confirming the behavioral sensitization to each drug and the cross-sensitization between cocaine and ethanol.

mRNA expression of histone deacetylases in the prefrontal cortex following cocaine and/or ethanol sensitization

The mRNA levels of HDAC1 [challenge effect: $F(2, 42) = 7.462$, $P = 0.003$, Fig. 3a; challenge effect: $F(2, 42) = 8.149$, $P = 0.002$, Fig. 3b] and HDAC2 [challenge effect: $F(2, 42) = 5.551$, $P = 0.010$, Fig. 3c; challenge effect: $F(2, 42) = 8.356$, $P = 0.002$, Fig. 3d], two isoforms of class I HDACs were changed significantly by acute challenge with cocaine in the prefrontal cortex. Post hoc tests revealed that acute cocaine challenge decreased the mRNA expression of HDAC1 ($P = 0.003$, S-S vs. S-C, Fig. 3a; $P = 0.001$, S-S vs. C-C, Fig. 3a; $P = 0.049$, S-S vs. E-C, Fig. 3b) and HDAC2 ($P = 0.009$, S-S vs. S-C, Fig. 3c; $P = 0.016$, S-S vs. C-C, Fig. 3c; $P = 0.021$, S-S vs. E-C, Fig. 3d), whereas ethanol challenge did not affect the mRNA expression of HDAC1 ($P = 0.842$, S-S vs. S-E, Fig. 3a; $P = 0.438$, S-S vs. C-E, Fig. 3a; $P = 0.546$, S-S vs. E-E, Fig. 3b) and HDAC2 ($P = 0.720$, S-S vs. S-E, Fig. 3c; $P = 0.652$, S-S vs. C-E, Fig. 3c; $P = 0.664$, S-S vs. E-E, Fig. 3d). Neither cocaine (C-S) nor ethanol (E-S) sensitization alone altered the expression of either HDAC compared to corresponding naïve (S-S) state (HDAC1: $P = 0.558$, S-S vs. C-S, Fig. 3a; $P = 0.650$, S-S vs. E-S, Fig. 3b; HDAC2: $P = 0.801$, S-S vs. C-S, Fig. 3c; $P = 0.867$, S-S vs. E-S, Fig. 3d). In short, the two isoforms of class I HDACs was not affected by the sensitization but acutely responded to cocaine challenge.

The mRNA levels of HDAC4 [sensitizing effect: $F(2, 42) = 78.964$, $P < 0.001$, Fig. 3e; sensitizing effect: $F(2, 42) = 59.083$, $P < 0.001$, Fig. 3f] and HDAC5 [sensitizing effect: $F(2, 42) = 33.143$, $P < 0.001$, Fig. 3g; sensitizing effect: $F(2, 42) = 29.936$, $P < 0.001$, Fig. 3h], two isoforms of class II HDACs, changed significantly following sensitization to cocaine and ethanol in the prefrontal cortex. Post hoc tests revealed that the mRNA levels of HDAC4 ($P = 0.001$, S-S vs. C-C, Fig. 3e; $P = 0.001$, S-S vs. C-E, Fig. 3e; $P = 0.006$, S-S vs. E-C, Fig. 3f; $P = 0.003$, S-S vs. E-E, Fig. 3f) and HDAC5 ($P = 0.008$, S-S vs. C-C, Fig. 3g;

$P = 0.010$, S-S vs. C-E, Fig. 3g; $P = 0.006$, S-S vs. E-C, Fig. 3h; $P = 0.018$, S-S vs. E-E, Fig. 3h) were not altered by acute cocaine or ethanol challenge despite sensitization status. However, the sensitization itself reduced both mRNAs compared to corresponding naïve states (HDAC4: $P < 0.001$, S-S vs. C-S, Fig. 3e; $P = 0.001$, S-S vs. E-S, Fig. 3f; HDAC5: $P = 0.003$, S-S vs. C-S, Fig. 3g; $P = 0.008$, S-S vs. E-S, Fig. 3h). Thus, the two isoforms of class II HDACs were affected by the sensitization but not by the acute challenge with drugs.

mRNA expression of HDACs in the dorsal striatum following either or both of cocaine and ethanol sensitization

The mRNA level of HDAC1 in the dorsal striatum changed significantly following either acute or chronic treatment with cocaine and ethanol [sensitizing effect: $F(2, 42) = 5.648$, $P = 0.025$, Fig. 4a; challenge effect: $F(2, 42) = 8.003$, $P = 0.002$, Fig. 4b]. Post hoc tests revealed that acute challenge with cocaine (-C) decreased the mRNA expression of HDAC1 in the dorsal striatum compared to corresponding control (-S) in the naïve ($P = 0.021$, S-S vs. S-C, Fig. 4a) and ethanol-sensitized ($P = 0.032$, E-S vs. E-C, Fig. 4b) state but not in the cocaine-sensitized state ($P = 0.513$, C-S vs. C-C, Fig. 4a). This may be because cocaine sensitization itself already reduced the HDAC1 mRNA ($P = 0.048$, S-S vs. C-S, Fig. 4a). The cocaine (-C) and ethanol (-E) challenge had little effect on HDAC1 mRNA levels in the cocaine-sensitized animals ($P = 0.954$, C-S vs. C-E, Fig. 4a). Neither acute ethanol (-E) challenge nor ethanol-induced sensitization (E-) had an effect on the mRNA expression of HDAC1 ($P = 0.915$, S-S vs. S-E; $P = 0.650$, S-S vs. E-E, Fig. 4b, respectively).

The effects of acute and chronic drug treatment on HDAC2 mRNA levels in the dorsal striatum were similar to those found in the prefrontal cortex [challenge effect: $F(2, 42) = 6.595$, $P = 0.005$, Fig. 4c; challenge effect: $F(2, 42) = 4.046$, $P = 0.031$, Fig. 4d]. That is, acute cocaine (-C) challenge decreased the mRNA expression of HDAC2 ($P = 0.004$, S-S vs. S-C, Fig. 4c; $P = 0.040$, C-S vs. C-C, Fig. 4c; $P = 0.031$, E-S vs. E-C, Fig. 4d) in both sensitized and naïve animals (C-, E-, S-); however, neither cocaine (C-) nor ethanol (E-) sensitization alone altered HDAC2 mRNA levels ($P = 0.903$, S-S vs. C-S, Fig. 4c; $P = 0.682$, S-S vs. E-S, Fig. 4d). The effects of acute and chronic ethanol (E-, -E) treatment on HDAC2 mRNA levels were not significantly different from those of the corresponding controls ($P = 0.769$, S-S vs. S-E; $P = 0.379$, S-S vs. E-E, Fig. 4d, respectively).

The mRNA levels of HDAC4 [sensitizing effect: $F(2, 42) = 35.507$, $P < 0.001$, Fig. 4e; sensitizing effect: $F(2, 42) = 48.976$, $P < 0.001$, Fig. 4f] and HDAC5 [sensitizing effect: $F(2, 42) = 19.015$, $P < 0.001$, Fig. 4g; sensitizing effect: $F(2, 42) = 24.245$, $P < 0.001$, Fig. 4h] in the dorsal striatum were similar to those in the prefrontal cortex.

Post hoc tests revealed that the mRNA levels of HDAC4 ($P = 0.748$, C-S vs. C-C, Fig. 4e; $P = 0.551$, C-S vs. C-E, Fig. 4e; $P = 0.756$, E-S vs. E-C, Fig. 4f; $P = 0.980$, E-S vs. E-E, Fig. 4f) and HDAC5 ($P = 0.373$, C-S vs. C-C, Fig. 4g; $P = 0.890$, C-S vs. C-E, Fig. 4g; $P = 0.877$, E-S vs. E-C, Fig. 4h; $P = 0.776$, E-S vs. E-E, Fig. 4h) were not altered by cocaine (-C) or ethanol (-E) challenge; however, both cocaine (C-) and ethanol (E-) sensitization reduced them (HDAC4: $P = 0.002$, S-S vs. C-S, Fig. 4e; $P = 0.001$, S-S vs. E-S, Fig. 4f; HDAC5: $P = 0.002$, S-S vs. C-S, Fig. 4g; $P = 0.002$, S-S vs. E-S, Fig. 4h). In short, the patterns of changes in HDAC mRNA levels induced by cocaine and ethanol treatments were similar in the prefrontal cortex and dorsal striatum with the exception that changes in HDAC1 expression induced by cocaine sensitization were observed only in the dorsal striatum.

Discussion

Our findings suggest that behavioral sensitization induced by different addictive drugs may share common cellular mechanisms related to the epigenetic control of behavioral phenotypes. This commonality would underly cross-sensitization: individuals sensitized to one type of drug exhibit sensitized behavior to another type of drug with no previous exposure to that drug. Our main findings were that the mRNA levels of class II HDACs (HDAC4 and HDAC5) were attenuated in the prefrontal cortex and dorsal striatum of animals sensitized to cocaine or ethanol after repeated treatment. The effect of cocaine sensitization on the expression of class II HDACs is well known: chronic cocaine treatment decreases the nuclear export of HDAC5 and histone hyperacetylation, which are associated with enhanced behavioral responsiveness [37,38]. Furthermore, chronic cocaine treatment has been shown to decrease the mRNA levels of HDAC4 in the nucleus accumbens, which contributes to the maintenance of addictive behaviors [25,39]. Here, we report the first evidence that ethanol-induced behavioral sensitization decreases class II HDAC mRNA levels. The epigenetic regulation of gene expression by altering HDAC expression may explain why behavioral sensitization or addictive behaviors induced by abusive drugs persist long after drug-use ceases.

It is worth noting that while several studies have shown that the acute administration of HDAC inhibitors enhances the locomotor response to psychoactive drugs [22–25], others have reported that the chronic administration of HDAC inhibitors suppresses behavioral sensitization to addictive drugs [27,28]. This discrepancy can be explained by different durations of drug treatment. HDAC inhibitors increase global histone acetylation and thereby promote gene expression. Chronic administration of an HDAC inhibitor may activate homeostatic processes to compensate for the already heightened transcriptional activity and behavioral response. We found

that chronic, but not acute, administration of addictive drugs decreased the mRNA levels of class II HDACs in the prefrontal cortex and dorsal striatum. These findings suggest that cocaine and ethanol are not themselves regulators of HDACs, but rather HDAC regulation is the final integrated neural response to repeated drug use. This systemic response leads to the downregulation of HDAC and behavioral sensitization, which is unlikely to be further regulated by homeostatic feedback, resulting in persistent behavioral change.

Although locomotor activity was less pronounced in the ethanol sensitization paradigm than in the cocaine sensitization paradigm, the decrease in the expression of class II HDACs was not significantly different between the drugs. This finding suggests that the HDAC response was not a consequence of repeated locomotor activity; rather, it was related to the common mechanism underlying sensitization. Alcohol consumption enhances compulsive cocaine self-administration by mediating the degradation of HDAC4 and HDAC5 in the nucleus accumbens [40], suggesting that cross-sensitization, at the level of drug-use behavior, is related to the downregulation of class II HDACs; our findings are consistent with this notion. However, in some experimental paradigms, the sensitization-related behaviors of HDAC4 and HDAC5 were different from each other; for example, repeated treatment with cocaine promoted dephosphorylation and nuclear localization of HDAC5 in the striatum while it promoted phosphorylation and cytoplasmic localization of HDAC4 [38,41]. Thus, specific roles of each isoform in drug-induced sensitization should be investigated further.

We found that the mRNA levels of HDAC1/2 class I HDAC isoforms decreased in the prefrontal cortex and dorsal striatum following the acute administration of cocaine regardless of sensitization status. This finding is consistent with that of a previous study, which found that the inhibition of class I HDACs contributed to the enhanced behavioral response to acute cocaine treatment [42]. Thus, cocaine may be an acute regulator of class I HDACs; however, the behavioral correlation is less clear. Cocaine-induced sensitization itself decreased HDAC1 level; thus, the acute challenge did not further decrease the isoform levels. Moreover, in the sensitized state, HDAC1 expression was not altered by cocaine challenge, whereas the behavioral response markedly increased.

A previous study found that HDAC1 mRNA levels increased in the mouse striatum after acute or repeated (10 injections) ethanol administration (1 or 2 g/kg) [43]. By contrast, another study found that global HDAC activity was reduced after acute administration of ethanol (2 g/kg) [44]. While our findings showing that repeated ethanol treatment reduced class II HDACs levels and acute ethanol treatment had no effect are not consistent with

those results, this disparity may be explained by methodological differences. We focused on the sensitizing effect of ethanol, and the ethanol dose administered (0.5 g/kg) was significantly lower than those of the previous studies. At this lower dose, symptoms of acute ethanol intoxication, that is, lethargy and loss of the righting reflex were absent, and the behaviors of the rats in our study were grossly normal.

In conclusion, we found that HDAC expression was dynamically regulated by cocaine- or ethanol-induced behavioral sensitization in the rat prefrontal cortex and dorsal striatum. The acute behavioral response to cocaine was accompanied by the downregulation of class I HDACs, whereas behavioral sensitization was associated with the downregulation of class II HDACs. Thus, the role-player is shifted from class I to class II with the development of sensitization. The specific changes in the mRNA expression of the HDAC 4/5 isoforms in the prefrontal cortex and dorsal striatum suggest that a common epigenetic mechanism may be involved in behavioral sensitization induced by a variety of substances, including cocaine and ethanol.

Acknowledgements

This work was supported by the National Research Foundation of Korea (NRF) grant (NRF-2019R1H1A2080237 to U.G.K. and NRF-2019R1C1C1008342 to S.X.) funded by the Ministry of Science and ICT, South Korea. Support was also provided by the KBRI basic research program (21-BR-03-03 to J.W.K.).

Conflicts of interest

There are no conflicts of interest.

References

- Robinson TE, Becker JB. Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. *Brain Res* 1986; **396**:157–198.
- Robinson TE, Berridge KC. The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res Brain Res Rev* 1993; **18**:247–291.
- Wise RA, Bozarth MA. A psychomotor stimulant theory of addiction. *Psychol Rev* 1987; **94**:469–492.
- Post RM, Rose H. Increasing effects of repetitive cocaine administration in the rat. *Nature* 1976; **260**:731–732.
- Pierce RC, Kalivas PW. A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants. *Brain Res Brain Res Rev* 1997; **25**:192–216.
- Paulson PE, Camp DM, Robinson TE. Time course of transient behavioral depression and persistent behavioral sensitization in relation to regional brain monoamine concentrations during amphetamine withdrawal in rats. *Psychopharmacology (Berl)* 1991; **103**:480–492.
- Itzhak Y, Martin JL. Effects of cocaine, nicotine, dizocipiline and alcohol on mice locomotor activity: cocaine-alcohol cross-sensitization involves upregulation of striatal dopamine transporter binding sites. *Brain Res* 1999; **818**:204–211.
- Lett BT. Repeated exposures intensify rather than diminish the rewarding effects of amphetamine, morphine, and cocaine. *Psychopharmacology (Berl)* 1989; **98**:357–362.
- Vezina P, Giovino AA, Wise RA, Stewart J. Environment-specific cross-sensitization between the locomotor activating effects of morphine and amphetamine. *Pharmacol Biochem Behav* 1989; **32**:581–584.
- Vezina P, Stewart J. Amphetamine administered to the ventral tegmental area but not to the nucleus accumbens sensitizes rats to systemic morphine: lack of conditioned effects. *Brain Res* 1990; **516**:99–106.
- Beyer CE, Stafford D, LeSage MG, Glowa JR, Steketee JD. Repeated exposure to inhaled toluene induces behavioral and neurochemical cross-sensitization to cocaine in rats. *Psychopharmacology (Berl)* 2001; **154**:198–204.
- Xu S, Kang UG. Characteristics of ethanol-induced behavioral sensitization in rats: Molecular mediators and cross-sensitization between ethanol and cocaine. *Pharmacol Biochem Behav* 2017; **160**:47–54.
- Xu S, Kang UG. Behavioral cross-sensitization between cocaine and ethanol is accompanied by parallel changes in the activity of AMPK system. *Pharmacol Biochem Behav* 2019; **183**:32–37.
- Feinberg AP. Phenotypic plasticity and the epigenetics of human disease. *Nature* 2007; **447**:433–440.
- Tsankova N, Renthal W, Kumar A, Nestler EJ. Epigenetic regulation in psychiatric disorders. *Nat Rev Neurosci* 2007; **8**:355–367.
- Grewal SI, Moazed D. Heterochromatin and epigenetic control of gene expression. *Science* 2003; **301**:798–802.
- Kumar A, Choi KH, Renthal W, Tsankova NM, Theobald DE, Truong HT, *et al.* Chromatin remodeling is a key mechanism underlying cocaine-induced plasticity in striatum. *Neuron* 2005; **48**:303–314.
- Grunstein M. Histone acetylation in chromatin structure and transcription. *Nature* 1997; **389**:349–352.
- Hsieh J, Gage FH. Chromatin remodeling in neural development and plasticity. *Curr Opin Cell Biol* 2005; **17**:664–671.
- Kouzarides T. Chromatin modifications and their function. *Cell* 2007; **128**:693–705.
- Volmar C-H, Wahlestedt C. Histone deacetylases (HDACs) and brain function. *Neuroepigenetics* 2015; **1**:20–27.
- Kalda A, Heidmets LT, Shen HY, Zharkovsky A, Chen JF. Histone deacetylase inhibitors modulates the induction and expression of amphetamine-induced behavioral sensitization partially through an associated learning of the environment in mice. *Behav Brain Res* 2007; **181**:76–84.
- Shen HY, Kalda A, Yu L, Ferrara J, Zhu J, Chen JF. Additive effects of histone deacetylase inhibitors and amphetamine on histone H4 acetylation, cAMP responsive element binding protein phosphorylation and DeltaFosB expression in the striatum and locomotor sensitization in mice. *Neuroscience* 2008; **157**:644–655.
- Sun J, Wang L, Jiang B, Hui B, Lv Z, Ma L. The effects of sodium butyrate, an inhibitor of histone deacetylase, on the cocaine- and sucrose-maintained self-administration in rats. *Neurosci Lett* 2008; **441**:72–76.
- Wang L, Lv Z, Hu Z, Sheng J, Hui B, Sun J, Ma L. Chronic cocaine-induced H3 acetylation and transcriptional activation of CaMKIIalpha in the nucleus accumbens is critical for motivation for drug reinforcement. *Neuropsychopharmacology* 2010; **35**:913–928.
- Schroeder FA, Penta KL, Matevosian A, Jones SR, Konradi C, Tapper AR, Akbarian S. Drug-induced activation of dopamine D(1) receptor signaling and inhibition of class I/II histone deacetylase induce chromatin remodeling in reward circuitry and modulate cocaine-related behaviors. *Neuropsychopharmacology* 2008; **33**:2981–2992.
- Kim WY, Kim S, Kim JH. Chronic microinjection of valproic acid into the nucleus accumbens attenuates amphetamine-induced locomotor activity. *Neurosci Lett* 2008; **432**:54–57.
- Romieu P, Host L, Gobaille S, Sandner G, Aunis D, Zwiller J. Histone deacetylase inhibitors decrease cocaine but not sucrose self-administration in rats. *J Neurosci* 2008; **28**:9342–9348.
- Malvaez M, Sanchis-Segura C, Vo D, Lattal KM, Wood MA. Modulation of chromatin modification facilitates extinction of cocaine-induced conditioned place preference. *Biol Psychiatry* 2010; **67**:36–43.
- Di Chiara G, Imperato A. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc Natl Acad Sci U S A* 1988; **85**:5274–5278.
- Steketee JD. Cortical mechanisms of cocaine sensitization. *Crit Rev Neurobiol* 2005; **17**:69–86.
- Wise RA. Roles for nigrostriatal—not just mesocorticolimbic—dopamine in reward and addiction. *Trends Neurosci* 2009; **32**:517–524.
- Pascual M, Do Couto BR, Alfonso-Loeches S, Aguilar MA, Rodriguez-Arias M, Guerri C. Changes in histone acetylation in the prefrontal cortex of ethanol-exposed adolescent rats are associated with ethanol-induced place conditioning. *Neuropharmacology* 2012; **62**:2309–2319.
- Renthal W, Nestler EJ. Histone acetylation in drug addiction. *Semin Cell Dev Biol* 2009; **20**:387–394.
- National Research Council (NRC). *Guide for the Care and Use of Laboratory Animals*. Eighth Edition. Washington, National Academies Press, 2010.

- 36 Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 2001; **25**:402–408.
- 37 Renthall W, Maze I, Krishnan V, Covington HE 3rd, Xiao G, Kumar A, *et al.* Histone deacetylase 5 epigenetically controls behavioral adaptations to chronic emotional stimuli. *Neuron* 2007; **56**:517–529.
- 38 Taniguchi M, Carreira MB, Smith LN, Zirlin BC, Neve RL, Cowan CW. Histone deacetylase 5 limits cocaine reward through cAMP-induced nuclear import. *Neuron* 2012; **73**:108–120.
- 39 Sando R 3rd, Gounko N, Pieraut S, Liao L, Yates J 3rd, Maximov A. HDAC4 governs a transcriptional program essential for synaptic plasticity and memory. *Cell* 2012; **151**:821–834.
- 40 Griffin EA Jr, Melas PA, Zhou R, Li Y, Mercado P, Kempadoo KA, *et al.* Prior alcohol use enhances vulnerability to compulsive cocaine self-administration by promoting degradation of HDAC4 and HDAC5. *Sci Adv* 2017; **3**:e1701682.
- 41 Penrod RD, Carreira MB, Taniguchi M, Kumar J, Maddox SA, Cowan CW. Novel role and regulation of HDAC4 in cocaine-related behaviors. *Addict Biol* 2018; **23**:653–664.
- 42 Kennedy PJ, Feng J, Robison AJ, Maze I, Badimon A, Mouzon E, *et al.* Class I HDAC inhibition blocks cocaine-induced plasticity by targeted changes in histone methylation. *Nat Neurosci* 2013; **16**:434–440.
- 43 Legastelois R, Botia B, Naassila M. Blockade of ethanol-induced behavioral sensitization by sodium butyrate: descriptive analysis of gene regulations in the striatum. *Alcohol Clin Exp Res* 2013; **37**:1143–1153.
- 44 Botia B, Legastelois R, Alaux-Cantin S, Naassila M. Expression of ethanol-induced behavioral sensitization is associated with alteration of chromatin remodeling in mice. *PLoS One* 2012; **7**:e47527.