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The histone deacetylase inhibitor sodium butyrate modulates acquisition and extinction of cocaine-induced conditioned place preference

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Abstract

Despite decades of research on treatments for cocaine dependence, relapse rates following many behavioral and drug-based therapies remain high. This may be in part because cocaine-associated cues and contexts can invoke powerful drug cravings years after quitting. Recent studies suggest that drugs that promote cognitive function can enhance the formation of memories involving cocaine and other substances. One target of these drugs is facilitating histone acetylation to promote learning by increasing gene transcription that supports memory formation. Here, we investigate the effects of the histone deacetylase (HDAC) inhibitor sodium butyrate (NaBut) on cocaine-induced conditioned place preference (CPP) in C57BL/6 mice. After establishing a graded dose-response curve (2, 5, & 20 mg/kg) for cocaine-induced CPP, we examined the effects of different doses of NaBut (0, 0.3, 0.6, & 1.2 g/kg) on conditioning, extinction, and post-extinction reconditioning of CPP. A high dose of NaBut (1.2 g/kg) enhanced initial acquisition of cocaine CPP, but there were no effects of NaBut on reconditioning of extinguished CPP. Effects of NaBut on extinction were more complex, with a low-dose (0.3 g/kg) facilitating extinction and a high dose (1.2 g/kg) weakening extinction evident by preference at a retention test. These findings suggest that HDAC inhibition may have dose dependent effects on different components of cocaine CPP, with implications for (1) involvement of histone acetylation in context-drug learning, (2) interpretation of acute and chronic drug effects, and (3) the targeting of different types of learning in therapeutic application of HDAC inhibitors.

Keywords

Epigenetics; Addiction; Cocaine; Drug-Seeking; Histone Acetylation

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Introduction

Cocaine abuse is a major public health issue. Relapse rates following many therapies remain high, in part because drug-associated cues and contexts can invoke powerful cravings years after quitting (Dutra et al., 2008; O'Brien et al., 1998; Penberthy et al., 2010; Volkow et al., 2008). During extinction, the relation between a drug and context is severed, decreasing drug seeking without loss of the original memory. Application of targeted pharmacological manipulations during or following exposure therapy may strengthen extinction of cue-induced cravings (Liddie et al., 2012; Nic Dhonnchadha and Kantak, 2011). Several recent studies have demonstrated that enhancing histone acetylation during consolidation of extinction can promote the development and persistence of extinction (Hui et al., 2010; Malvaez et al., 2010; Pastor et al., 2011; Romieu et al., 2011; Schroeder et al., 2008; Wang et al., 2010). These findings are important, because a key challenge in facilitating extinction of drug conditioning is to make the change in behavior long-lasting, as drug-seeking often returns with time or is reinstated by stressors or drug-conditioned cues (Feltenstein and See, 2008; Reichel and Bevins, 2009).

Learning is critically modulated by a dynamic interaction between histone acetylation and deacetylation (Malvaez et al., 2009; Malvaez et al., 2011; McQuown and Wood, 2010; Renthall and Nestler, 2009; Stafford et al., 2012). For example, inhibiting histone deacetylases (HDACs) increases histone acetylation, which facilitates access to genomic DNA by transcription factors such as CREB (Candido et al., 1978; Davie, 2003; Vecsey et al., 2007) by prolonging the time period that chromatin structure is open and available for transcription following cell signaling events (McQuown and Wood, 2011). Histone acetylation and increased transcription of CREB-regulated genes during memory consolidation has been associated with enhancement of various tasks, such as acquisition and extinction of fear conditioning (Bredy and Barad, 2008; Bredy et al., 2007; Dash et al., 2009; Fischer et al., 2007; Itzhak et al., 2012; Lattal et al., 2007; Levenson et al., 2004), novel object recognition (Guan et al., 2009; Hawk et al., 2011; McQuown et al., 2011; Stefanko et al., 2009), and extinction of conditioned place preference (Malvaez et al., 2010).

HDAC inhibitors, such as sodium butyrate (NaBut) or SAHA (Suberoylanilide hydroxamic acid) have generated excitement because of the potential of these compounds as small molecule therapeutics for a variety of cognitive and neurodegenerative disorders (Abel and Zukin, 2008; Malvaez et al., 2009; McQuown and Wood, 2010; Robison and Nestler, 2011; Zovkic and Sweatt, 2013). In the case of extinction, this is particularly relevant to disorders that involve failures of inhibition, such as substance abuse and post-traumatic stress disorder, where one goal of treatment is to promote learning that occurs during extinction-based exposure therapies (Kaplan and Moore, 2011; Stafford and Lattal, 2011).

Although an emerging literature demonstrates the power of HDAC inhibitors in facilitating extinction, some issues remain unresolved. First, although there are several demonstrations of HDAC inhibitors enhancing extinction, these studies often have examined a single dose of drug. Thus, little is known about the dose dependency of these effects. It is important to characterize dose effects because there is evidence that some compounds can increase responding following an extinction session rather than decrease it (Bredy and Barad, 2008; Lee et al., 2006; Tronson and Taylor, 2007). Second, no studies have examined the effects of HDAC inhibition on relapse. This is important because clinical application of cognitive enhancing drugs could have the unintended effect of enhancing learning following periods of relapse after extinction. By investigating the effects of NaBut on conditioning, extinction, and reconditioning after extinction, we will begin to address the effects of HDAC inhibition on different learning processes.

The following experiments examine the dose-effects of the HDAC inhibitor NaBut on cocaine-induced conditioned place preference after (1) initial formation of a context-drug memory, (2) extinction of drug-context seeking, and (3) reconditioning of extinguished drug-context associations. Additionally, because dose effects have been difficult to show in place preference (Bardo and Bevins, 2000), we characterize a dose-response function of cocaine on CPP. Collectively, our results suggest that acquisition and extinction of drug-seeking are dose-dependently modulated by NaBut.

Results

Experiment 1: Cocaine-Induced CPP is Graded and Dose-Responsive

Cocaine's dose response curve for establishing CPP was investigated by treating animals with 2, 5, or 20 mg/kg cocaine over four CS+ and four CS- trials followed by a preference test, the results of which are shown in Figure 1. ANOVA showed that dose of cocaine at conditioning had a significant effect on CS+ time [$F(2,56) = 8.8$, $p < 0.0005$; Figure 1A]. Post-hoc tests demonstrated that both 20 and 5 mg/kg doses generated more preference than did 2 mg/kg, and that preference in 20 mg/kg treated mice was greater than that of 5 mg/kg. Additionally, to demonstrate linearity of these effects, data are plotted as log doses alongside a linear function (Figure 1A). Examination of preference (G+ vs G-) within each treatment condition showed that while 20 mg/kg [$t(21) = 6.2$, $p < 0.00005$] and 5 mg/kg [$t(19) = 2.5$, $p < 0.05$] cocaine produced preference, 2 mg/kg did not (Figure 1B).

Experiment 2: NaBut Enhances Initial Conditioning of Cocaine-Induced CPP

To examine effects of NaBut on CPP conditioning, mice were conditioned with a lower dose of cocaine (5 mg/kg) with fewer pairings (three CS+ trials followed by injections of NaBut (0, 0.3, 0.6, or 1.2 g/kg) and three CS- trials followed by vehicle injection). This reduction in trial number with a low dose of CPP should result in only a moderate preference developing in vehicle-treated animals, which allows NaBut-induced enhancements to be detected. ANOVA showed an effect of NaBut treatment on CS+ time [$F(3,57) = 4.2$, $p < 0.01$; Figure 2A]. Post-hoc tests showed that 1.2 g/kg NaBut enhanced preference compared to all other treatment groups, suggesting that a high-dose of NaBut enhanced acquisition of weak CPP (Figure 2A).

Examination of preference (G+ vs G-) within treatment conditions showed that mice treated with 0.3 or 1.2 g/kg NaBut showed significant CPP [0.3 g/kg, $t(15) = 3.0$, $p < 0.01$; 1.2 g/kg, $t(15) = 6.0$, $p < 0.00005$; Figure 2B].

Experiment 3: NaBut Modulates Extinction of Cocaine-Induced CPP

To investigate the effects of NaBut on extinction, mice were first conditioned with two CS+ 20 mg/kg cocaine, and CS- saline trials. Twenty-four hours later preference was assessed (Preference in Table 1). Mice were then assigned to NaBut treatment groups, and received 4 extinction sessions (EXT1-4) followed by NaBut (0, 0.3, 0.6, 1.2 g/kg) administration (Figure 3). Mice then received an additional preference test (EXT5) followed by reconditioning. One (Reconditioning) and 21 days (Retention) after reconditioning mice were again assessed for preference (Figure 4).

Extinction—Figure 3 shows that preference in each dose decreased over the course of extinction. Repeated-measures ANOVA showed that across extinction there was a main effect of session [$F(1,46) = 33.9$, $p = 0.0000005$], but no drug effects and no interaction. Closer examination of preference (G+ vs G-) within each dose during extinction (Table 1) showed that all groups stopped expressing a preference by the final extinction session, but that this occurred sooner in subjects treated with 0.3 or 0.6 g/kg NaBut.

Reconditioning and Retention—Figure 4 shows that reconditioning was impaired in animals that received 0.3 mg/kg NaBut during extinction. An ANOVA on CS+ time following reconditioning (Figure 4A) showed an effect of NaBut [$F(3,44) = 3.8$, $p = 0.0165$], and post-hoc tests showed that mice treated with 0.3 g/kg NaBut after extinction showed less reconditioned preference compared to vehicle-treated controls, suggesting that this low-dose enhanced extinction resulting in greater resistance to reconditioning. Within dose preference (G+ vs G-) was evident in 0 mg/kg [$t(15) = 3.63$, $p = 0.0025$] and 1.2 mg/kg NaBut [$t(15) = 2.46$, $p = 0.027$] treated mice but not in the other groups (Figure 4B). ANOVA of preference (CS+) at a retention test 3 weeks following reconditioning showed an effect of NaBut [$F(3,44) = 2.8$, $p < 0.05$; Figure 4C]. Post-hoc tests showed no difference between vehicle-treated mice and mice treated with any dose of NaBut. Instead, mice treated with 0.3 g/kg NaBut showed significantly less preference than mice treated with 1.2 g/kg NaBut, suggesting that NaBut may have had biphasic effects on extinction, with a low dose showing a trend towards facilitating extinction and a high dose showing a trend towards producing deficits. Additionally, examination of within group preference (G+ vs G-) during the retention test (Figure 4D), showed that mice treated with vehicle or moderate doses (0.3 or 0.6 g/kg) of NaBut during extinction showed no preference, whereas mice treated with 1.2 g/kg NaBut showed place preference [$t(15) = 4.2$, $p < 0.001$].

Overall, these data suggest that NaBut may have had differential effects on consolidation of CPP extinction, with low doses facilitating and a higher dose potentially interfering with long-term consolidation of extinction under these experimental conditions.

Experiment 4: NaBut Does Not Affect Reconditioning of Extinguished Cocaine CPP

To investigate the effects of NaBut on post-extinction reconditioning, mice were trained and extinguished in CPP as described in Experiment 3 except that mice were not assigned to NaBut treatment conditions until reconditioning. During reconditioning mice received 3 pairs of weak CS+ (5 mg/kg, cocaine) and CS- trials, with each CS+ trial followed by NaBut (0.0, 0.3, 0.6, 1.2 g/kg) administration. ANOVA failed to show a significant effect of drug on post-reconditioning preference (Figure 5A). Additionally, examination of preference (G+ vs G-) within treatment conditions showed that all groups exhibited significant place preference [0.0, $t(15) = 3.5$, $p < 0.005$; 0.3, $t(15) = 2.1$, $p < 0.05$; 0.6 $t(15) = 4.2$, $p < 0.005$; 1.2, $t(15) = 7.5$, $p < 0.00001$; Figure 5B].

Experiment 5: NaBut Enhances Histone Acetylation

To examine the dose effects of NaBut administration on histone acetylation in brain regions involved in acquisition and extinction of CPP, we examined H3K14 acetylation in IL and NAc, following NaBut administration (Figure 6). Two-way within-subject ANOVA of IL and NAc histone acetylation positive nuclei divided by total DAPI-stained nuclei, with region as the within subject measure, showed a main effect of NaBut dose [$F(1,13) = 9.3$, $p = 0.01$] and a dose by region (IL or NAc) interaction [$F(1,13) = 6.2$, $p = 0.05$].

Discussion

These experiments demonstrate that (1) HDAC inhibition can facilitate initial conditioning of cocaine-induced CPP; (2) HDAC inhibitors, such as NaBut, may have dose-dependent effects on extinction of cocaine-induced CPP, with moderate doses generating extinction that is resistant to reconditioning relative to vehicle, and a high dose of NaBut causing poor long-term retention of extinction; (3) that cocaine-induced CPP is both dose-responsive and graded, making it a good assay for detecting changes in drug-context learning. The effects of different doses of HDAC inhibitor on conditioning and the differential effects of high and

low doses on extinction suggest that the effects of HDAC inhibition on memory consolidation are complex and may be mediated by multiple mechanisms.

HDAC inhibition & CPP extinction

The finding that NaBut (0.3 g/kg) enhances extinction is consistent with other studies reporting that NaBut can enhance learning at lower doses (Hui et al., 2010). Although the general finding of an extinction enhancement induced by NaBut is consistent with the literature, most studies have examined effects of 1.2 g/kg NaBut (Lattal et al., 2007; Malvaez et al., 2010; Wang et al., 2010), which in our hands actually produced weaker extinction than a lower dose, as revealed through poor long-term retention of extinction. This could reflect enhancement of reconsolidation of drug-seeking by NaBut, similar to that reported on fear conditioning (Bredy and Barad, 2008), or a blockade of extinction, but the theoretical mechanisms underlying these effects remain unknown.

Time course of extinction and NaBut effects

Malvaez et al. (2010) reported enhancement of extinction of cocaine-induced CPP by 1.2 g/kg NaBut. We found that NaBut did not strongly affect behavior during extinction trials, but a low-dose (0.3 g/kg) facilitated extinction to produce greater resistance to reconditioning and a trend in retention. There are a number of differences between the current study and that of Malvaez et al (2010) that may be responsible for the difference in the effects of the high dose of NaBut on extinction. For instance, the protocol used by Malvaez et al (2010) generated preference that began to extinguish with a single trial, and NaBut facilitated extinction such that very little preference was evident at a second test, whereas in the present studies preference in vehicle-treated mice remained across many trials and effects of NaBut were not evident until later trials.

The timing and frequency of NaBut administration in relation to learning may partially explain this difference. NaBut inhibits class I HDACs 1, 2, 3, and 8 (Kilgore et al., 2010; Lahm et al., 2007), but the effects of repeated NaBut administration on learning are not well characterized. For instance, 28 days of daily NaBut (1.2 g/kg) administration has been shown to enhance acquisition of contextual fear conditioning (Fischer et al., 2007), but treatment with the same dose for 21 days has been shown to have no effect (Kilgore et al., 2010). This disparity suggests complex effects of NaBut over time. It may be that the effects of repeated NaBut administration differ from its effects following a single administration, as has been reported with other cognitive-enhancing drugs such as nicotine (Der-Avakian and Markou, 2010; Epping-Jordan et al., 1998; Raybuck and Gould, 2009; Solomon and Corbit, 1973). In fact, in neuronal cell cultures, 12 and 24 h incubation with HDAC inhibitor phenyl-butyrate enhanced expression of HDACs 2, 3, & 5 (Ajamian et al., 2004), which negatively regulate spatial and drug-cue learning (Guan et al., 2009; McQuown et al., 2011; Renthall et al., 2007). However, whether upregulation occurs in vivo remains to be seen. Such effects could generalize to other HDAC inhibitors and may be important to understand in clinical application of HDAC inhibitors for cognitive therapies.

Additionally, there are other reports of different effects of NaBut on learning (Kilgore et al., 2010; Levenson et al., 2004). Thus, the effects of HDAC inhibitors on behavior may reflect larger issues with use of NaBut to enhance learning as well as effects of cognitive enhancers in general, since effects of NaBut vary markedly across laboratories and tasks (Bredy and Barad, 2008; Lattal et al., 2007; Pastor et al., 2011; Romieu et al., 2011; Stefanko et al., 2009). It is possible, for example, that small changes in neural mechanisms supporting the task, strength of learning, or drug-administration route and timing may shift the dose curve of this drug, generating findings that seem divergent, but rather reflect complex effects. Indeed, other cognitive enhancing drugs, such as nicotine, show dose by task interactions.

For instance, effects of dorsal hippocampal nicotine infusion vary with task difficulty. Infusion of 0.35 ug/side enhances weak contextual fear learning. However, with stronger conditioning 0.35 ug is no longer effective, and enhancement occurs at 0.09 ug/side, one quarter the dose (Raybuck and Gould, 2010). Similar effects have been shown for NaBut on extinction of contextual fear, where NaBut effects interact with the protocols used to induce conditioning and extinction (Stafford et al., 2012). Thus, dose effects of cognitive enhancing drugs can interact with task demands, and through this interaction drugs that exert inverted U-shaped effects may produce false negatives, generating seemingly conflicting findings. More thorough investigation of dose-effects of HDAC inhibitors and other cognitive enhancing compounds may help to explain null effects under some conditions and enhancing effects under others (Stafford and Lattal, 2011; Stafford et al., 2012).

The idea that HDAC inhibitors interact with learning induced histone acetylation fits well with the present data demonstrating that NaBut enhances histone acetylation in IL and NAc, though only weak effects were seen at lower doses. It is likely the case that the present dose effects of NaBut on learning are caused by an interaction between NaBut-facilitated and learning-induced acetylation (e.g. Stafford et al., 2012). Thus, the present behavioral effects of lower doses of NaBut on extinction may reflect that low doses, though not increasing histone acetylation on their own, could add to the effects of learning induced histone acetylation, whereas it is possible that higher doses have such strong effects on histone acetylation that they obscure learning-induced changes in histone acetylation.

The present data combined with existing literature demonstrate that HDAC inhibition can modulate both conditioning and extinction of CPP (Malvaez et al., 2010; Malvaez et al., 2011), but the brain regions in which NaBut may be facilitating gene expression to drive these extinction effects are not yet clear. Previous studies have used convergent pharmacological and genetic techniques to demonstrate the importance of striatum and nucleus accumbens (NAc) in CPP, specifically that NaBut enhances histone acetylation in the striatum and NAc (Kumar et al., 2005; Malvaez et al., 2010), and that genetic manipulation of creb-binding protein (CBP) activity in the NAc alters CPP (Malvaez et al., 2011). Thus, there is a clear role for this reward structure in the epigenetic modulation of drug-cue learning. However, the role of histone acetylation in the many structures that support CPP is largely unknown. Further investigation will be necessary to determine how regional histone acetylation modulates extinction of CPP.

HDAC Inhibition and Reconditioning following Extinction

One implication of the present findings is that effects of HDAC inhibition on initial conditioning and reconditioning following extinction (which may reflect processes that occur in humans relapsing to drug use) may be different. Importantly, the present studies suggest that it is possible to dissociate these effects by dose, as a low-dose (0.3 g/kg) that enhanced extinction had no effect on conditioning or reconditioning, and a high-dose (1.2 g/kg) enhanced conditioning but interfered with extinction. These findings suggest that similar dissociation may be possible in therapeutic application. As this class of compounds has been considered as potential cognitive enhancers to facilitate exposure therapy in treatment of both drug abuse and anxiety disorders (Abel and Zukin, 2008; Stafford and Lattal, 2011; Zovkic and Sweatt, 2013), it will be important to consider dose effects of these drugs to maximize effects on extinction and minimize chances that a period of relapse paired with HDAC inhibition could enhance the long-term effects of relapse.

The present experiments, together with existing literature demonstrate that enhancement of histone acetylation via inhibition of HDACs can modulate learning, but that these effects are dynamic, differing based on drug-dose, task, and behavioral protocol. It may be possible to exploit these complexities to facilitate application of this class of drugs as cognitive

enhancers in clinical settings. Future studies should seek to further characterize the dose effects of HDAC inhibitors on learning, how these compounds interact with task difficulty, and how administration route and timing may alter drug effects.

Materials and Methods

Animals

These experiments were conducted using 272, male, 8–12 week old C57BL6/J mice (Jackson Laboratories, Bar Harbor, ME), housed 4 per cage with *ad lib* food and water. All procedures were conducted during the light phase of a regular 12-hour light/dark cycle, approved by the Oregon Health & Science University Institutional Animal Care and Use Committee, and in accordance with the ethical guidelines of the National Institutes of Health and the Society for Neuroscience.

Drugs

Cocaine HCl (Sigma-Aldrich, St. Louis, MO) was dissolved in saline and administered intraperitoneally (ip) at 2, 5, or 20 mg/kg (10 ml/kg) prior to CPP conditioning sessions. 1M NaBut (Millipore, Temecula, California) was diluted in distilled water and administered ip postsession at 0, 0.3, 0.6, or 1.2 g/kg (1.2 ml/kg). In all cases Nabut was administered post-session, minimizing possible effects of the drug on conditioning or preference expression. Additionally, the variety of effects reported here and elsewhere strongly suggests that NaBut modulates CPP by affecting consolidation processes following learning, rather than direct effects of the drug on behavior (Lattal et al., 2007; Malvaez et al., 2010; Stafford et al., 2012).

Apparatus

The conditioning apparatus consisted of four plexiglas (33 × 18 × 15cm) enclosures with interchangeable hole and grid floors (Cunningham et al., 2006). Conditioning chambers were housed in sound-attenuating cubicles, equipped with infrared LED illuminators and B/W CCD video cameras. Behavioral data were analyzed using Ethovision XT 5 software (Noldus Technology, Leesburg, Virginia).

Experimental Procedures

General conditioning—An unbiased CPP procedure was adapted from Bernardi and Lattal (2010). Briefly, mice were first habituated to the CPP apparatus, during which animals were weighed, injected with vehicle (ip) and then placed in the sound-attenuating chamber on a white paper floor for 5 min. Animals within each treatment condition were then randomly assigned to counterbalanced conditioning subgroups that received either a grid (G+) or hole-floor (G−) paired with cocaine (+) and the other paired with saline (−). Mice were conditioned over consecutive days with daily, alternating CS+ and CS− sessions. During conditioning, animals were injected with either cocaine (+) or saline (−) then placed in the conditioning apparatus for 15 min. Thus, G+ treated mice received cocaine on the grid floor and saline on the hole floor on alternate days; G− treated mice received cocaine on the hole floor and saline on the grid floor on alternate days. This resulted in counterbalanced subgroups (G+/G−) within each experimental treatment group (e.g., dose of NaBut). Twenty-four hours following the final conditioning session, mice were placed in the test apparatus with both floors (grid and hole) for a 15-min test session. In some experiments mice received repeated preference tests that served as extinction sessions (choice extinction). Locomotor activity and time spent on each floor was recorded across all sessions.

Experiment 1: Effects of Cocaine Dose on CPP—Mice received four CS+ and four CS– pairings over 8 days of conditioning, followed by a preference test 24 hours later. Cocaine doses were 2, 5, or 20 mg/kg.

Experiment 2: Effects of NaBut of Conditioning of Cocaine CPP—Based on Experiment 1, mice were conditioned with the lowest effective dose of cocaine (5 mg/kg) and only three conditioning trials to facilitate detection of effects of NaBut against a low baseline. Mice received a total of three CS+ (cocaine) and three CS– (saline) trials intermixed. Each CS+ trial was followed by administration of NaBut (0.0, 0.3, 0.6, or 1.2 g/kg), followed 24 hours later by a preference test.

Experiment 3: Effects of NaBut on Extinction of Cocaine CPP—Mice received 2 CS+ (20 mg/kg cocaine) and 2 CS– (saline) trials, followed 24 hours later by a preference test. This experiment used a higher dose of cocaine to generate a moderate preference that would leave room on the scale to see potential enhancements in extinction (weakening of the preference) or enhancements in reconsolidation (strengthening of the preference). Preference during this test was used to assign drug (NaBut) conditions, resulting in four groups with matched pre-extinction preferences. Over the next 4 days, mice received preference tests (EXT1–4), each of which was followed by NaBut (0.0, 0.3, 0.6, or 1.2 g/kg) administration, followed 24 hours later by an additional preference test (EXT5). Mice did not receive NaBut following EXT5 because there would be no way to assess drug effects without an additional preference test. Mice then received reconditioning sessions with a single CS+ (paired with 5 mg/kg cocaine) and a single CS– trial on two consecutive days (NaBut was not administered during reconditioning). The day after the last reconditioning session, mice received an additional test session (Reconditioning), followed by a long-term retention test (Retention) 21 d later.

Experiment 4: Effects of NaBut on Reconditioning of Cocaine CPP—As in Experiment 2 mice received a total of three CS+ (20 mg/kg cocaine) and three CS– (saline) trials. Because the goal of this experiment was to detect effects of NaBut on post-extinction reconditioning, we conducted more extensive extinction sessions than in Experiment 3 (i.e., 7 choice extinction sessions). NaBut was not administered during conditioning or extinction. Following extinction, mice received reconditioning trials identical to the conditioning trials in Experiment 2, with NaBut (0.0, 0.3, 0.6, or 1.2 g/kg) administered following the CS+ trial.

Experiment 5: Immunofluorescence Effects of NaBut on Histone Acetylation—To examine changes in histone acetylation (HA) induced by NaBut, mice were administered systemic NaBut (0.0, 0.3, 0.6, or 1.2 g/kg), and one hour later tissue was collected for fluorescent immunohistochemistry (IHC). Tissue was flash frozen, cut at 20µm on a cryostat, thaw-mounted on plus-coated slides, fixed for 10m in 4% PFA-PBS, blocked in 8% NGS PBSt for 1hr, washed, incubated in primary antibody for acetylation of lysine 14 on histone 3 (H3K14ac, 06-911, Millipore, Billerica, MA, 1:1000), washed, incubated 2hr with secondary anti-rabbit FITC-conjugated antibody (1:1000, Vector Laboratories, Burlingame, CA), washed, and cover-slipped with DAPI-containing Vectashield (Vector Laboratories). All images were collected under equal conditions and analyzed with ImageJ macros optimized for thresholded nucleus counts within regions of interest, allowing semi-quantitative analysis of HA. In these studies, images of infralimbic cortex (IL) and nucleus accumbens (NAc) were collected at 20x from the same section. Analysis was conducted on total (DAPI-stained) nuclei, and on ratio of H3-AC positive nuclei/DAPI-labeled nuclei. No differences in total nuclei per ROI/subject were evident. Data are presented as ratio HA positive nuclei as percent vehicle control.

Data Analysis

Preference was assessed in two ways. First, to detect dose effects, preference was calculated as seconds/minute spent on CS+ during testing, reported as dose curves for drug effects on place preference. Effects on preference were examined with one-way ANOVA, followed by Tukey's Post-Hoc. Homogeneity of variance was confirmed with Levene's Test. In Experiment 3, preference across extinction trials was analyzed using a repeated-measures ANOVA with trial as the repeated measure. Second, simple planned-comparisons between G+ and G- subgroups within each dose were conducted to determine whether animals expressed preference within individual treatment conditions. These data are reported as time on grid floor split by Grid+ (Grid paired with cocaine) and Grid- (grid paired with saline) conditioning subgroups (Cunningham, et al. 2006). In Experiment 5 (IHC), data were analyzed with a 2-way ANOVA. Alpha was held at 0.05 for all analyses. Analysis of locomotor activity showed direct effects of cocaine administration on locomotion during training, but no effects of NaBut condition on locomotion (data not shown).

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Highlights

We establish that cocaine-induced conditioned place preference is dose-responsive and graded.

The histone deacetylase inhibitor sodium butyrate promotes acquisition of cocaine-induced conditioned place preference at high doses.

The same drug promotes extinction at moderate doses and impairs extinction at higher doses.

Figure 1. Cocaine-induced CPP is dose-responsive and graded

(A) CS+ data plotted as log doses demonstrate a clear, linear, dose-response curve for cocaine-induced CPP. 20 mg/kg cocaine produced stronger place preference compared to 5 or 2 mg/kg CPP. Data are mean time on CS+ $\pm$ SEM, trend line fitted to group means. (B) Comparison of time on Grid floor in Grid (G+) vs Hole (G-) conditioned mice showed that 5 and 20, but not 2 mg/kg cocaine induced significant place preference. Thus, low-doses of cocaine induce weaker CPP. Data are mean $\pm$ SEM of time on grid for grid (G+) and hole (G-) conditioned mice, $n = 20$ –24. Asterisks denote significant difference between comparison groups.

Figure 2. NaBut enhances acquisition of cocaine-induced CPP

Administration of NaBut following CPP conditioning sessions facilitated CPP acquisition.

(A) CS+ data show that 1.2 g/kg NaBut significantly enhanced acquisition of cocaine-CPP compared to all other groups. (B) Time on grid across treatment conditions shows that place preference was induced in mice treated with 0.3 or 1.2 g/kg NaBut but not 0 or 0.6 g/kg, suggesting that NaBut enhanced acquisition of CPP, $n = 16$. Data are from a preference test conducted after three CS+ and CS- conditioning trials. Data are mean $\pm$ SEM; asterisks denote significant difference between comparison groups.

Figure 3. NaBut does not alter the development of extinction of CPP

Across extinction sessions, preference decreased in all groups and administration of NaBut following each session had no effect on subsequent preference. Data are mean $\pm$ SEM.

Figure 4. NaBut alters the persistence of extinction of CPP

(A) Following post-extinction reconditioning, a preference test revealed a significant effect of drug on CS+ time, with 0.3 g/kg NaBut administered following extinction sessions 1–4 generating extinction that was resistant to reconditioning compared to vehicle controls. (B) Examination of time on Grid floor between training conditions (G+ & G–) showed that vehicle and 1.2 g/kg NaBut treated mice reacquired preference following reconditioning, but mice treated with 0.3 or 0.6 g/kg NaBut were resistant to reconditioning, suggesting NaBut enhanced extinction at these doses. (C) CS+ data show that NaBut following extinction had long-term effects on preference 3-weeks following reconditioning. Mice treated with 0.3 g/kg NaBut showed less preference than mice treated with 1.2 g/kg. (D) Analysis of time on Grid floor shows that only mice treated with 1.2 g/kg NaBut following extinction show preference at retention, suggesting that while lower doses enhanced extinction, the high dose of NaBut may have impaired long-term retention of CPP extinction. Data are mean $\pm$ SEM; asterisks denote significant difference between comparison groups, $n = 14$ – 16 .

Figure 5. NaBut administered during post-extinction reconditioning does not alter CPP
(A) Administration of NaBut following reconditioning had no significant effect on cocaine place preference. (B) Examination of time on Grid floor between G+ and G- training conditions showed that all mice reacquired cocaine place preference. Data are mean $\pm$ SEM; asterisks denote significant difference between comparison groups, $n = 16$.

Figure 6. NaBut induces histone acetylation in infralimbic cortex and nucleus accumbens
(A) NaBut administration enhances histone acetylation (H3K14). Data are Mean $\pm$ SEM of ratio of histone acetylation positive nuclei to DAPI-stained nuclei, presented as percent vehicle (0.0 g/kg NaBut) control. **(B)** Representative micrographs of histone acetylation immunofluorescence in infralimbic cortex (IL) and nucleus accumbens (NAc) show clear induction of histone acetylation by NaBut.

Table 1

NaBut effects on extinction of cocaine-CPP

Analysis of preference expression across Experiment 3 showed that preference was extinguished faster in mice treated with lower doses of NaBut and that mice in these groups were also resistant to reconditioning of cocaine-CPP. Mice treated with the high-dose of NaBut showed slower extinction of preference than low dose treated mice and were resistant to reconditioning, but showed resumed preference at a 3-week retention test. Data are (Mean sec/min on grid floor, SEM), highlighted cells indicate loss of preference indicated by a non-significant t-test between G+ and G– within each dose.

NaBut (g/kg)	Training Condition	Preference	EXT1	EXT2	EXT3	EXT4	EXT5	Post-Recon
0.0	G+	34, 2.9	34, 1.6	34, 3.1	36, 3.2	34, 2.0	35, 2.8	37, 1.3
	G–	20, 2.5	23, 2.2	27, 2.5	25, 1.7	26, 1.9	31, 2.3	28, 2.4
0.3	G+	38, 1.5	35, 1.5	36, 2.6	37, 3.2	33, 2.1	30, 2.8	32, 3.7
	G–	25, 1.5	22, 1.9	27, 2.0	26, 3.1	30, 2.5	30, 1.8	33, 1.7
0.6	G+	36, 0.6	35, 1.7	36, 3.0	32, 2.6	30, 2.7	32, 2.6	31, 3.3
	G–	23, 2.8	25, 0.5	28, 0.9	28, 1.4	28, 0.6	32, 1.0	28, 1.8
1.2	G+	34, 2.7	36, 1.3	36, 1.6	38, 1.1	33, 0.7	31, 3.2	37, 1.6
	G–	21, 0.6	22, 1.1	25, 1.8	26, 1.1	25, 1.4	30, 1.2	27, 1.7

Mean, SEM (**Highlight** indicates lack of preference (sig t-test between G+ & G–, n = 8).