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Exposure to histone deacetylase inhibitors during Pavlovian conditioning enhances subsequent cue-induced reinstatement of operant behavior

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Abstract

Histone deacetylase inhibitors (HDACIs) strengthen memory following fear conditioning and cocaine-induced conditioned place preference. Here, we examined the effects of two non-specific HDACIs, valproic acid (VPA) and sodium butyrate (NaB), on appetitive learning measured via conditioned stimulus (CS)-induced reinstatement of operant responding. Rats were trained to lever press for food reinforcement and then injected with VPA (50–200 mg/kg, i.p.), NaB (250–1000 mg/kg, i.p.), or saline vehicle (1.0 ml/kg), 2h before receiving pairings of noncontingent presentation of food pellets preceded by a tone+light cue CS. Rats next underwent extinction of operant responding followed by response-contingent re-exposure to the CS. Rats receiving VPA (100 mg/kg) or NaB (1000 mg/kg) prior to conditioning displayed significantly higher cue-induced reinstatement than did saline controls. Rats that receiving either vehicle or VPA (100 mg/kg) prior to a conditioning session with a randomized relation between presentation of food pellets and the CS failed to show subsequent cue-induced reinstatement with no difference between the two groups. These findings indicate that, under certain contexts, HDACIs strengthen memory formation by specifically increasing the associative strength of the CS, not through an increasing motivation to seek reinforcement.

Keywords

histone deacetylase inhibitors; learning; reinforcement; reinstatement

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Conflicts of Interest

There are no conflicts of interest.

Introduction

Learning, histone acetylation and deacetylation

It is well established that the formation of long-term memories require gene expression, which is regulated by epigenetic processes (Day and Sweatt, 2011). Chromatin modification represents one such epigenetic mechanism that is critically involved in experience-dependent plasticity. Generally speaking, acetylation of specific lysine residues on histone tails contributes to gene activation, whereas deacetylation promotes chromatin compaction, leading to gene silencing (for reviews, see e.g. Berger, 2007; Borrelli et al., 2008; Maeshima and Eltsov 2008). Histone acetylation is regulated by a number of enzymes including histone acetyltransferases, CREB-binding protein (CBP), and p300/CBP-associated factor, which add acetyl groups and promote a relaxed chromatin state that is permissive for gene expression. Conversely, histone deacetylases (HDACs) including those belonging to the class I and II families of HDACs remove acetyl groups from histone tails leading to chromatin compaction, which generally represses transcriptional activity. There are a variety of commercially available pharmacological compounds that directly inhibit histone acetyltransferases and HDACs, thereby allowing investigation into the role of these particular histone modifications in behavioral adaptations and psychiatric disorders (Meinhold et al. 2005; Michaelis et al. 2007; Ghaemi et al. 2007; Bredy et al. 2010).

HDACs have been implicated in many aspects of neural plasticity underlying learning and memory (see e.g. Morris et al., 2010; McQuown and Wood, 2011). For example, systemic administration of HDAC inhibitors (HDACIs) strengthen memory for contextual (Levenson et al. 2004) and cued (Bredy et al., 2007; Lattal et al., 2007; Bredy and Barad, 2008) fear. HDACIs also enhance memory for cocaine-induced conditioned place preference when administered either systemically (Kumar et al., 2005) or via intra-nucleus accumbens (Renthall et al., 2007). Although there are previous studies examining the effect of HDACIs on operant responding (Romieu et al., 2008; Sun et al., 2008), they have yielded mixed results and the effect of HDAC inhibition on the ability of conditioned reinforcers to control operant behavior has not yet been explored.

Histone deacetylase inhibitors and conditioned reinforcement

Stimuli paired explicitly with a reward come to control goal-directed behavior, such as operant responding, through a process called conditioned reinforcement. For instance, presentation of a conditioned stimulus (CS) paired with delivery of a reinforcer enables the CS to subsequently renew operant responding following extinction training (e.g. Kelleher and Gollub, 1962; Rescorla, 1988). Here, we have examined the effect of two distinct HDACIs, sodium butyrate (NaB) and valproic acid (VPA), on the strength of the association formed during conditioning as measured by the level of CS-induced reinstatement of extinguished operant responding using a design based upon the work of Kruzich and See (2001). This paradigm offers three major advantages over procedures previously used to study the impact of HDACIs on learning. First, operant response rates can be dissociated from general activity and operant responding is not required during the Pavlovian conditioning procedure, allowing for a highly specific assessment of the impact of the HDACIs on associative processes, thus minimizing the potential effects of HDACIs on

motor or motivational processes. Second, the contiguity of the CS and the reinforcer can be readily manipulated, and conditioned responses can be dissociated from motivation levels [as measured by progressive ratio (PR) responding]. Third, the ability of HDACIs to modulate response reinforcement can be studied to evaluate potential motivational and behavioral effects of these drugs independent of their effects on acquisition of associative conditioning. Additionally, the employment of food pellets as an unconditioned stimulus (UCS) represents a departure from similar studies which employed either foot shock or non-contingent drug exposure (both of which have aversive properties; see e.g. Mutschler and Miczek, 1998; Mantsch and Goeders, 2000) and allows a better procedure to examine the impact of HDACIs on appetitive conditioning processes. Here, we provide evidence that contingent, but not random, pairing of a CS with a reinforcer in the presence of HDAC inhibition allows the CS to have greater control over subsequent operant behavior independent of any effect on the motivation to seek reinforcement.

Methods

Subjects

Male Sprague–Dawley rats (weighing 275–300 g at the start of the experiment) were obtained from a commercial source (Charles-River, Wilmington, MA, USA). Rats were individually housed for 3–5 d prior to the start of the experimental procedures in a temperature- and humidity-controlled vivarium on a 12-h light–dark cycle (lights on at 7:00 a.m.). The housing and care of the rats followed the “Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research” (National Research Council 2003). During the course of the experiment, rats were restricted to 20g of rat chow (Harlan, Indianapolis, IN, USA) per day but had *ad libitum* access to water. All behavioral analyses were conducted during the light portion of the light-dark cycle.

Apparatus

All experimental procedures were conducted in operant chambers equipped with two retractable levers, a stimulus light above each lever, a food pellet (45 mg) dispenser between the levers, a white house light on the wall opposite to the levers, and a speaker connected to a tone generator contained in sound-attenuated operant chambers (Med Associates, St. Albans, VT, USA). Rats remained in the colony between daily experimental sessions. Rats were always tested in the same chamber and the chambers were cleaned thoroughly with a 30% ethanol solution and bedding was replaced prior to the start of each session.

Procedures: Effects of VPA and NaB during Pavlovian Conditioning on Subsequent Conditioned Cue-Induced Reinstatement

Rats ($n = 10 - 12$ per drug group) were trained to lever press according to a fixed-interval 20 s (FI20) schedule of food reinforcement. Responses on the “active” (right) lever were reinforced with a single 45 mg food pellet, whereas responses on the “inactive” (left) lever had no programmed consequences. Daily sessions were terminated when a maximum of 25 food pellets were obtained or when 1 h had transpired. Once rats obtained 25 pellets for two consecutive sessions, a time-out period (TO) following delivery of each food pellet was introduced that was initially 10 s and then increased to 20 s for 3 consecutive sessions.

Following acquisition of operant behavior, each rat underwent a Pavlovian (explicitly-paired) conditioning session under the influence of either vehicle (1.0 ml/kg saline, i.p.), VPA (50, 100, 200 mg/kg, i.p.), or NaB (250, 500, or 1000 g/kg, i.p.) injected 2 h prior to the start of the conditioning session; rats were assigned to drug conditions such that the mean number of active lever responses during the preceding FI20 session was equivalent between groups. The conditioning session lasted 1 h during which the levers were retracted and each rat received 25 pairings of a 5 s presentation of the CS (right stimulus light+tone) immediately followed by delivery of a food pellet; trials were spaced evenly across 60 min (i.e. inter-trial interval of 144 s). After conditioning was completed, rats were returned to their home cages for 48 h and then received 3 additional FI20 food-reinforced operant behavior sessions.

To investigate the associative nature of the impact of HDACIs during conditioning on subsequent cue induced-reinstatement, separate groups of rats (n = 12 per group) were trained to respond for food reinforcement then exposed to either vehicle or 100 mg/kg VPA 2 h prior to exposure to randomly paired presentations of food and the (light+tone) CS. Specifically, the delivery of the food pellet was identical to that in the explicitly-paired condition above except that the CS was presented at random times throughout the 1 h conditioning session in order to produce exposure to both the CS and US in a completely non-contingent manner (Rescorla, 1967). After random conditioning, rats received an additional 3 sessions of food reinforcement as above.

Next, all rats underwent extinction training during which they were placed in the operant chambers with both “active” and “inactive” levers extended, however responding had no programmed consequences. Rats were required to undergo a minimum of five 1 h extinction sessions and achieve the extinction criterion of 25 or fewer responses on the “active” lever for two consecutive sessions, with the average responses on the final two extinction sessions serving as the “extinction baseline” for each rat.

After rats completed extinction training, they received a cue-induced reinstatement of food-seeking behavior test. At the beginning of the test, each rat was placed in the chamber with both levers extended and a 5 s non-contingent presentation of the CS (light+tone). Following the initial presentation of the CS, active lever responses resulted in additional CS presentations under a FI20 schedule, whereas inactive lever responses resulted in no programmed consequences.

Procedures: Effect of VPA and NaB on food-seeking under fixed interval and progressive ratio (PR) schedules of reinforcement

Male rats were trained to press the right lever according to a FI20 schedule of food reinforcement during daily 30-min sessions. After the completion of at least 5 sessions of FI20 and once the number of reinforcers that a rat achieved during each session had less than 20% variability over 3 FI20 session, one cohort (n = 12) received three counterbalanced tests preceded by a vehicle or HDACI (100 mg/kg VPA or 1000 mg/kg NaB) injection 2 h prior to the FI20 test sessions (followed by 1 day off and 2 additional FI20 sessions prior to the next test). A second cohort of rats (n = 16) were trained on a FI20 schedule as above and then on a progressive ratio schedule of food reinforcement during which a reinforcer was

delivered following completion of an incrementally increasing number of lever responses (with the progression of increments following that of Richardson and Roberts, 1996) with a period of 30 min between successive reinforcers resulting in termination of the test session. Once the number of reinforcers that a rat achieved during each session had less than 20% variability over 3 PR sessions, they received three counterbalanced tests preceded by a vehicle or HDACI (100 mg/kg VPA or 1000 mg/kg NaB) injection 2 h prior to PR test sessions (with re-establishment of the stability criterion between each test and the average number of reinforcers achieved during the sessions preceding each test serving as a baseline for each rat). A third cohort of rats ($n = 18$) were trained on FI20 and PR schedules as above and then received three tests that were preceded by injections of vehicle, 50 mg/kg, or 200 mg/kg VPA each given 2 h prior to one of the tests with the order of each injection presented in a counterbalanced fashion. Finally, for some of the rats ($n = 16$) baseline was again re-established (i.e. less than 20% variability across 3 PR sessions) and rats were subjected to a 48 h food deprivation period followed by a PR test session and then allowed *ad libitum* access to food in the home-cage for 48 h followed by a final PR test session.

Drugs

VPA (50, 100, or 200 mg/kg, i.p.; Sigma, St. Louis, MO), NaB (250, 500, or 1000 mg/kg, i.p.; Sigma, St. Louis, MO), were dissolved in 0.9% bacteriostatic saline and administered in 1.0 ml/kg, *i.p.* Doses were selected based on prior studies employing these drugs to modify learning or neural plasticity (e.g. Bredy et al., 2007; Bredy and Barad, 2008; Kalda et al., 2007) and administered 2 h prior to treatment sessions (conditioning or operant; see below) based on previous reports indicating that peak histone acetylation occurred at this time-point after injection (Tremolizzo et al., 2002).

Statistical Analysis

Responses on the active or inactive levers and numbers of reinforcers were analyzed by separate mixed-factor analyses of variance (ANOVAs) with drug treatment serving as a between-subjects factors and session serving as a within-subjects factor. Separate ANOVAs were used to compare responding under food reinforcement, extinction of operant behavior, cue-induced reinstatement of operant behavior, and PR schedule of food reinforcement. Significant interactions were analyzed by simple effect analyses followed by comparison of individual means Tukey's post-hoc tests (Glass and Hopkins 1984) as appropriate. The level of statistical significance for all comparisons was 0.05.

Results

HDACI during Pavlovian conditioning increases subsequent cue-induced reinstatement of operant behavior

VPA delivered prior to the Pavlovian conditioning session dose-dependently produced higher active lever responding during subsequent cue-induced reinstatement but did not alter the numbers of pellets consumed during the Pavlovian conditioning sessions (all rats consumed all pellets). Similarly, VPA during conditioning did not alter responding during subsequent food reinforcement or extinction sessions (Figure 1).

All rats rapidly acquired the lever press response to obtain food reinforcement, and treatment with VPA did not alter subsequent active lever responding under FI20 or during extinction (all $ps > 0.05$). Moreover, a mixed factor ANOVA for active lever responding during the extinction baseline and the cue test revealed a significant interaction between dose and test session ($F_{3,44} = 3.98, p < 0.05$). Simple main effect analyses revealed a significant effect of dose during the cue test ($F_{3,44} = 4.06, p < 0.05$), with the 100 mg/kg VPA group responding significantly more than the vehicle and 50 mg/kg VPA groups ($ps < 0.05$) but no other groups differed. Conversely, there was no significant effect of dose on the extinction baseline ($p > 0.05$). No significant interactions or effects were detected for inactive lever responding (Figure 1A).

Similarly, NaB delivered prior to the Pavlovian conditioning session did not alter the numbers of pellets consumed during the Pavlovian conditioning sessions (again, all rats consumed all pellets) and did not alter responding during subsequent food reinforcement or extinction sessions, but did produce higher responding during subsequent cue-induced reinstatement (Figure 1B). As above, all rats rapidly acquired the lever press response to obtain food reinforcement and treatment with NaB did not alter subsequent active lever responding under FI20 or during extinction (all $ps > 0.05$). Moreover, a mixed factor ANOVA for active lever responding during the extinction baseline and the cue test revealed a significant interaction between dose and test session ($F_{3,50} = 4.778, p < 0.05$). Simple main effect analyses revealed a significant effect of dose during the cue test ($F_{3,50} = 4.00, p < 0.05$) with the 1000 mg/kg NaB group responding significantly more than the vehicle, 250, or 500 mg/kg NaB groups ($ps < 0.05$) but no other groups differed. Conversely, there was no significant effect of dose on the extinction baseline ($p > 0.05$). No significant interactions or effects were detected for inactive lever responding (Figure 1B).

VPA (100 mg/kg) failed to alter cue-induced reinstatement when delivered before random pairing of the CS and food reinforcer (Figure 1C). Rats rapidly acquired the lever press response to obtain food reinforcement and VPA treatment did not alter subsequent active lever responding under FI20 or during extinction (all $ps > 0.05$). A between-within ANOVA of active lever responding during the extinction baseline and cue test revealed no significant effects or interactions; however, the effect of test was close to significant ($F_{1,22} = 4.26, p = 0.06$) with a trend to higher responding during the cue test. Further, during the cue test, the VPA group tended to have a slightly higher increase from the extinction than the vehicle group (mean $\pm$ SEMs change from baseline for VPA, $44\% \pm 28\%$; for vehicle, $36\% \pm 28\%$; $p > 0.05$), but these are substantially lower increases than observed with Pavlovian (paired) conditioning (see Figure 1A; mean $\pm$ SEMs change from baseline for VPA, $748\% \pm 156\%$; for vehicle, $293\% \pm 57\%$; $p < 0.05$). No significant interactions or effects were detected for inactive lever responding (Figure 1C).

HDACIs do not consistently alter food-reinforced operant responding

All rats acquired operant responding under FI20 schedule of food reinforcement and stabilized within 6 sessions. However, there were no effects of HDACIs on the number of reinforcers earned during the 30-min FI20 sessions (Figure 2A). A repeated measures ANOVA failed to detect significant effects of either VPA or NaB treatment relative to

vehicle ($F_{2,22} = 0.54$, $p > 0.05$). Further, no effects were observed on the number of responses made during the session ($F_{2,22} = 1.12$, $p > 0.05$; mean $\pm$ SEM for vehicle, 197.5 ± 36.4 ; for VPA, 220.3 ± 57.1 ; for NaB, 149.8 ± 30.5).

All rats also acquired operant responding under FI20 schedule of food reinforcement and exhibited stable progressive ratio (PR) responding in 10–14 sessions (mean $\pm$ SEM, 11.13 ± 0.19 sessions), as well as, immediate re-stabilization following all vehicle and HDACI challenges (i.e. no detectable carry-over effects; data not shown). However, there were inconsistent effects produced by the HDACIs on responding during the PR schedule of food reinforcement; whereas NaB reduced the number of reinforcers received, no effect of VPA was observed (Figure 2B; for simplicity vehicle data are combined from the two cohorts of rats in the graph). A repeated measures ANOVA comparing the numbers of pellets obtained under the PR reinforcement schedule for the cohort receiving vehicle, 100 mg/kg VPA, or 1000 mg/kg NaB revealed a significant effect of drug ($F_{2,30} = 10.79$, $p < 0.05$) with lower responding during the NaB test relative to the other conditions ($ps < 0.05$). Whereas, a repeated measures ANOVA comparing the numbers of pellets obtained under PR reinforcement schedule for the cohort receiving vehicle, 50 mg/kg VPA, or 200 mg/kg VPA failed to reach significance ($F_{2,34} = 0.45$, $p > 0.05$).

Conversely, responding under the PR schedule of food reinforcement was highly sensitive to changes in food motivation as revealed by the number of reinforcers obtained under food deprivation and satiation conditions. A repeated measures ANOVA comparing the number of reinforcers obtained under baseline, deprivation, or satiation conditions revealed a significant effect of condition ($F_{2,45} = 13.5$, $p < 0.05$) with the numbers of reinforcers obtained being highest under deprivation and lowest under satiation (breakpoints under all conditions differed significantly, $ps < 0.05$; mean $\pm$ SEMs for baseline were 10.1 ± 0.66 , for deprivation were 13.0 ± 0.69 , and for satiation were 8.17 ± 0.64).

Discussion

The main finding is that HDACIs, administered prior to conditioning, related to Pavlovian-instrumental transfer, increases cue-induced reinstatement of operant behavior after extinction training and, critically, these effects only impacted associative conditioning processes. Rats that received either VPA (100, but not 50 or 200, mg/kg, i.p.) or NaB (1000, but not 250 or 500, mg/kg, i.p.) before the repeated pairings of a CS with noncontingent delivery of food subsequently displayed greater operant behavior during response-contingent re-exposure to the CS relative to controls. The increase in cue-induced operant responding exhibited by these HDACI groups was specific to the period of CS re-exposure (i.e. no differences between groups during maintenance or extinction training). Furthermore, the present study demonstrates for the first time that HDAC inhibition does not alter the behavioral impact of neutral stimuli when these cues are presented in a randomly paired conditioning procedure. These data indicate that the observed group differences resulted from increased associative strength between the CS (light+tone) and the food US rather than by alterations in food reinforcement or extinction learning. Reinstatement was also specific to food-reinforced behavior as there was an increase in active lever responding in the absence of any effect on the inactive lever responding during cue tests. Inconsistent effects

of the two HDACIs were observed on operant responding under a progressive ratio schedule of food reinforcement indicating that changes in food motivation cannot account for the observed effects on associative learning produced by these two HDACIs. Accordingly, the present data provide evidence for increased CS-elicited operant responding following conditioning under the influence of HDAC inhibition.

NaB and VPA were tested for potential impact on conditioned reinforcement because they are both potent pharmacological inhibitors of multiple class I and II HDACs (Gurvich et al., 2004; Ehrenkaufer et al., 2007). However, both compounds have a variety of other effects. VPA increases levels of the inhibitory neurotransmitter GABA, as well as sensitizing GABA receptors, and is a common treatment for epilepsy and bipolar disorder (Cunningham et al., 2003; Reynolds et al., 2007). The failure to observe enhanced conditioning with the highest VPA dose is consistent with another report that doses above 100 mg/kg of VPA fail to enhance learning and that these doses can produce sedative effects (Bredy et al., 2007). In contrast, NaB has no effect on GABA and is currently a treatment for a variety of diseases including cancers (Kim et al., 2007; Provenzano et al., 2007). These differences in pharmacological profile may underlie the observed difference on food-reinforced responding under a PR schedule of reinforcement observed in the present study. Accordingly, given that both NaB and VPA significantly enhanced cue-induced reinstatement of operant behavior despite differences in other pharmacological properties, it is likely that the observed potentiation of cue-induced reinstatement is due to their shared effect of inhibiting HDACs.

Consistent with our interpretation that NaB and VPA administered prior to Pavlovian conditioning enhances subsequent cue-induced reinstatement, histone modifying enzymes (and the drugs that modify their activity) have been implicated in many kinds of learning and memory (for reviews see Levenson and Sweatt, 2006; Graff and Mansuy, 2008). Systemic HDACIs increase the acquisition of conditioned fear (Bredy and Barad, 2008; Fischer et al., 2007; Levenson et al., 2004; Lattal et al., 2007; Vecsey et al., 2007) and cocaine-induced conditioned place preference (Kumar et al., 2005; Renthal et al., 2007; Mu and Yu, 2007), as well as, facilitating the extinction of conditioned fear (Bredy et al., 2007; Bredy and Barad, 2008; Marek et al., 2011) and drug-induced conditioned place preference (Malvaez et al., 2010; Wang R., et al., 2010). Systemic HDACIs also enhance locomotor sensitization to cocaine (Kumar et al., 2005) and amphetamine (Kalda et al., 2007; Sanchis-Segura et al., 2009). Thus, there is a high degree of consistency in a range of studies that indicate HDAC inhibition increases the strength of Pavlovian conditioning, and that this effect on cognition can impact the ability of the CS to control subsequent operant behavior. Moreover, none of the prior studies examining the impact of HDACIs on Pavlovian conditioning for appetitive UCSs have employed procedures that can distinguish associative and non-associative processes. Accordingly, our finding that HDAC inhibition during randomly paired exposure to CS and UCS fails to alter cue-induced reinstatement, suggests that HDAC inhibition does not enhance the strength of appetitive conditioned responses by altering the processes elicited by CS or UCS exposure in isolation but rather, requires their association. Interestingly, rats subjected to random pairing of the CS and UCS exhibited a non-significant increase in responding (approximately 41% across groups) during the cue-induced reinstatement test, relative to the extinction baseline. This suggests that this procedure may be able to impart some conditioned significance that could be measured

under reinstatement testing. Similarly, random pairing conditioning produces poor, but detectable, conditioned responses when tested under conditions of Pavlovian-Instrumental transfer, an effect that has been argued to reflect associative learning because it is subject to blocking effects (Rescorla, 2000). Nonetheless, the findings support the idea that the ability of HDACIs to potentiate cue-induced reinstatement is mediated specifically through alteration of associative strength between the CS and UCS.

Notably, only one dose each of VPA and NaB facilitated learning indicating a narrow dose-response curve for these compounds to enhance learning. Despite the growing literature on the behavioral and molecular effects of HDACIs, there are relatively few studies addressing the dose-response nature of their effectiveness. The present study and Bredy et al (2007) are the only dose-response studies of the effects of VPA on learning; both studies found that 100 mg/kg VPA given IP at 2h prior to testing enhances learning (albeit in different paradigms) and both studies found that higher doses of VPA failed to enhance learning. This narrow effective range may indicate that the sedative effects of VPA at doses above 100 mg/kg (Tulloch et al., 1982; Loscher et al., 1989) counteract its memory enhancing properties. More attention has been directed at determining the dose-response relation of NaB to learning or behavioral plasticity than in VPA. Again, the results from the present study and Bredy et al (2007) indicate that 1000 mg/kg NaB administered IP 2h prior to testing enhances learning. This dose is similar to the most commonly employed dose of NaB used to enhance memory (e.g. Levenson et al., 2004; Lattal et al., 2007; Malvaez et al., 2010). In one study, the enhancing effects of a high dose of NaB was extended to a dose of 600 mg/kg delivered at 1 hour prior to an object memory training trial (Stefanko et al., 2009). Interestingly, Sanchis-Segura et al (2009) performed a partial dose-response curve which indicated that NaB can potentiate the behavioral plasticity induced by drugs of abuse at doses of 150 and 300 mg/kg at 20 min prior to drug exposure. This finding is supported by the results of Kumar et al., (2005) that indicate 200 mg/kg of NaB is ineffective in producing changes in H3 acetylation on its own but is additive to both the behavioral and molecular effects of cocaine. Accordingly, the present findings indicate that VPA has a narrow range as an effective cognitive enhancer and, although the effective range of NaB appears somewhat larger, it is still limited.

The lack of influence of NaB and VPA on responding during operant reinforcement supports the notion that HDAC inhibition specifically potentiates associative processes. NaB significantly reduced active lever responding during PR food reinforcement, whereas VPA had no effect. Although NaB and VPA are HDACIs, these compounds also have several different pharmacological effects that may explain the differences observed under the PR schedule of reinforcement. Further, administration of NaB was associated with the unexpected side-effects of transient immobility and writhing for approximately 15 min following injection (with no observable effects at 2 h post-injection) which may have contributed to the effect on subsequent food reinforced behavior. Importantly, during the Pavlovian conditioning experiment, NaB was given approximately 2 h prior to conditioning and 48 h prior to the next opportunity to respond for food, which may have negated any direct impact of the adverse side-effects on operant responding. Our finding that administration of NaB reduces PR responding is in contrast to evidence indicating that systemic administration of NaB (400 mg/kg) increased operant responding and intake of

cocaine under fixed ratio schedules of reinforcement (FR1 and FR5), but does not influence operant responding for sucrose (Sun et al., 2008). It is unclear if the differences in reported behavioral effects are due to the use of different reinforcers or different administration regimens (e.g. different doses, time for testing, etc). Intravenous administration of Trichostatin A (TSA), another potent HDACI, decreases cocaine self-administration, but has no effect on sucrose self-administration, under fixed or progressive ratio schedules (Romieu et al., 2008). Thus, operant behavior could be influenced by the involvement of specific HDAC is and these effects may be either dose or mediated by other off-target effects of the compounds tested. Conversely, selective class I HDACI's such as TSA or suberoylanilide hydrozamic acid (SAHA), infused directly into the nucleus accumbens shell increase cocaine intake under a PR schedule, whereas viral-mediated over-expression of HDAC4 in the same region decreases cocaine intake under a PR schedule (Wang, L., et al., 2010). Thus, further studies investigating the role of HDACs in regulating operant responding for food or other reinforcement are warranted, including those assessing regionally specific effects of HDAC manipulations and involvement of specific HDAC enzymes. Nonetheless, our findings indicate that the effects of systemic NaB and VPA on Pavlovian conditioning cannot be attributed directly to changes in motivation to seek reward.

In summary, the present study provides further evidence that systemic administration of HDACIs can increase the strength of associative learning and enhance memory. These effects are not mediated by increased motivation to seek reinforcement, as responding under a PR schedule of reinforcement was not consistently altered by HDACI nor are they mediated by the effects on non-associative processes, as responding for a randomly paired cue was not affected.

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Figure 1. Pharmacological inhibition of HDAC selectively increases the strength of CS control of operant responding

A. Active (left) and inactive (right) lever responding during food-reinforced operant sessions (FI20), extinction baseline (Ext), and conditioned cue-induced reinstatement of operant responding (Cue) in rats treated with vehicle or VPA (50, 100, or 200 mg/kg, IP) prior to Pavlovian conditioning. Rats treated with 100 mg/kg VPA exhibited significantly higher responding during cue-induced reinstatement than vehicle. **B.** Active (left) and inactive (right) lever responding during FI20, Ext, and Cue sessions in rats treated with vehicle or NaB (250, 500, 1000 mg/kg, i.p.) prior to Pavlovian conditioning. Rats treated with NaB exhibited significantly higher responding during cue-induced reinstatement than vehicle. **C.** Active (left) and inactive (right) lever responding during FI20, Ext, and Cue sessions in rats treated with vehicle or VPA (100 mg/kg, i.p.) prior to random pairing conditioning. No differences between groups were observed on responding. All data presented as mean + SEM; * $p < 0.05$ for comparison of drug group to vehicle. CS, conditioned stimulus; HDAC, histone deacetylase; NaB, sodium butyrate; VPA, valproic acid.

Figure 2. NaB, but not VPA, reduces operant responding under progressive ratio (PR), but not fixed interval 20 sec (FI20) schedules of food reinforcement

A. Reinforcers earned under a FI20 schedule of food reinforcement 2 hr following IP injection with vehicle, VPA (100 mg/kg), or NaB (1000 mg/kg) challenge. No significant effects were observed. **B.** Reinforcers earned under a PR schedule of food reinforcement 2 hr following injection with vehicle, VPA (50, 100, or 200 mg/kg), or NaB (1000 mg/kg) challenge. Only NaB reduced the number of reinforcers earned with no other significant differences between treatments. All data presented as mean + SEM; * $p < 0.05$ for comparison of drug treatment to vehicle. NaB, sodium butyrate; VPA, valproic acid.