Archival Report

Biological Psychiatry

# Habits Are Negatively Regulated by Histone Deacetylase 3 in the Dorsal Striatum

Melissa Malvaez, Venuz Y. Greenfield, Dina P. Matheos, Nicolas A. Angelillis, Michael D. Murphy, Pamela J. Kennedy, Marcelo A. Wood, and Kate M. Wassum

## ABSTRACT

**BACKGROUND:** Optimal behavior and decision making result from a balance of control between two strategies, one cognitive/goal-directed and one habitual. These systems are known to rely on the anatomically distinct dorsomedial and dorsolateral striatum, respectively. However, the transcriptional regulatory mechanisms required to learn and transition between these strategies are unknown. Here we examined the role of one chromatin-based transcriptional regulator, histone modification via histone deacetylases (HDACs), in this process.

**METHODS:** We combined procedures that diagnose behavioral strategy in rats with pharmacological and viral-mediated HDAC manipulations, chromatin immunoprecipitation, and messenger RNA quantification.

**RESULTS:** The results indicate that dorsal striatal HDAC3 activity constrains habit formation. Systemic HDAC inhibition following instrumental (lever press → reward) conditioning increased histone acetylation throughout the dorsal striatum and accelerated habitual control of behavior. HDAC3 was removed from the promoters of key learning-related genes in the dorsal striatum as habits formed with overtraining and with posttraining HDAC inhibition. Decreasing HDAC3 function, either by selective pharmacological inhibition or by expression of dominant-negative mutated HDAC3, in either the dorsolateral striatum or the dorsomedial striatum accelerated habit formation, while HDAC3 overexpression in either region prevented habit.

**CONCLUSIONS:** These results challenge the strict dissociation between dorsomedial striatum and dorsolateral striatum function in goal-directed versus habitual behavioral control and identify dorsostriatal HDAC3 as a critical molecular directive of the transition to habit. Because this transition is disrupted in many neurodegenerative and psychiatric diseases, these data suggest a potential molecular mechanism for the negative behavioral symptoms of these conditions and a target for therapeutic intervention.

*Keywords:* Chromatin, Decision making, Epigenetic, HDAC3, Instrumental conditioning, Learning, Reward

https://doi.org/10.1016/j.biopsych.2018.01.025

Humans and animals rely on two distinct strategies for decision making (1,2). The deliberative, goal-directed strategy requires prospective evaluation of potential actions and their anticipated consequences, via a learned association between these variables, and therefore supports behavior that can readily adapt when circumstances change (3). Repetition of successful actions promotes less cognitively taxing habits, in which behavior is more automatically triggered by antecedent events (4). The balance between these two forms of learning promotes adaptive and efficient behavior, but disruptions can lead to the symptoms that underlie myriad neurodegenerative and psychiatric diseases (5,6).

The goal-directed and habit strategies have been demonstrated to rely on largely distinct corticobasal ganglia circuits centered on the dorsomedial striatum (DMS) (7–10) and dorsolateral striatum (DLS) (11,12), respectively. Although gene transcription has been implicated (13–18), the mechanisms that regulate transcriptional events to support the acquisition of and transition between these behavioral control strategies are unknown. Posttranslational modifications of core histone proteins alter accessibility to DNA for transcriptional machinery to coordinate gene expression and have been implicated in the regulation of neuronal plasticity and memory (19–21), and thus could regulate goal-directed and/or habit learning. But the function of such mechanisms in instrumental learning or in the dorsal striatum is not known. Histone deacetylases (HDACs) are particularly interesting because they are removed from gene promoters after salient behavioral events, allowing histone acetyltransferases (HATs) to promote histone acetylation, which in turn allows transcriptional processes supporting the long-lasting changes in neuronal function that ultimately give rise to learning (22–24). Here we evaluated the role of HDACs in instrumental learning using procedures that diagnose behavioral strategy combined with pharmacological or viral-mediated manipulation of HDAC function and molecular analysis.

## METHODS AND MATERIALS

A detailed description of the methods is provided in the Supplement. Specific experiments are described below. All

SEE COMMENTARY ON PAGE 322

 

ISSN: 0006-3223

procedures were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the UCLA Institutional Animal Care and Use Committee.

## RESULTS

### Effect of Posttraining HDAC Inhibition on Instrumental Learning and Behavioral Strategy

We first examined the function of HDACs in goal-directed and habit learning by systemically inhibiting HDAC activity following instrumental conditioning and then probing the behavioral strategy off drug. Adult male rats were trained to lever press to earn delivery of a food-pellet reward on a random-interval 30-second reinforcement schedule. Rats were given either limited (3 days) or intermediate (4 days) training, known to preserve goal-directed control, or extended (6 days) training, known to allow habits to dominate (4) (Figure 1A). Rats were administered the nonspecific class I HDAC inhibitor sodium butyrate (25) (NaBut) [1.0 g/kg, intraperitoneal (26–30)] or sterile-water vehicle immediately after each training session to examine the function of HDACs in consolidation of the learning underlying instrumental performance. All rats acquired the behavior; both vehicle- and NaBut-treated rats increased their lever-press rate across training (Figure 1B–D) (training day: limited [$F_{3,60} = 92.59$, $p < .0001$], intermediate [$F_{4,80} = 50.69$, $p < .0001$], extended [$F_{6,96} = 18.64$, $p < .0001$]), though the NaBut group plateaued at a lower rate than the vehicle control group with limited (drug × day [$F_{3,60} = 3.63$, $p = .02$]) or intermediate ($F_{4,80} = 5.49$, $p < .001$) training, but not with extended ($F_{6,96} = 0.42$, $p = .86$) training.

Behavioral strategy cannot be determined from simple lever-press performance. To identify the degree of goal-directed versus habitual control, rats were given a 5-minute, drug-free, outcome-specific devaluation test (Figure 1A). Nonreinforced lever pressing was assessed following sensory-specific satiation (1-hour prefeeding) on the food pellet earned by lever pressing (devalued state). This was compared with pressing after satiation on an alternative food pellet that had been noncontingently provided daily outside of the training context (valued state). Each rat was tested under both conditions, and data were normalized between valued and devalued conditions to provide an index of the extent to which behavior was under goal-directed versus habitual control and to control for variability in press rates between subjects (for raw press rates, see Supplemental Figure S1). If subjects are using a goal-directed strategy and therefore considering the consequences of their actions, they should downshift pressing in the devalued state (sensitivity to devaluation). Habits are marked by insensitivity to devaluation. As expected, in vehicle-treated subjects we observed a reduction in lever pressing in the devalued state relative to the valued state in the limited training group (Figure 1E) (devaluation [$F_{1,20} = 19.68$, $p = .003$]) and a failure to reduce responding following devaluation in the extended training group (Figure 1G) ($F_{1,16} = 0.62$, $p > .999$). This was not altered by posttraining HDAC inhibition (drug: limited [$F_{1,20} = 0.00$, $p > .999$], extended [$F_{1,16} = 1.88$, $p = .19$]; drug × devaluation: limited [$F_{1,20} = 0.41$, $p = .53$], extended [$F_{1,16} = 0.06$, $p = .80$]), suggesting that HDAC inhibition neither disrupted initial goal-directed learning, nor prevented habit formation with overtraining. NaBut-treated subjects did show more variable performance following overtraining, which might have resulted from off-target effects of extended NaBut treatment.

Posttraining HDAC inhibition did, however, accelerate the rate at which habit came to dominate behavioral control. With

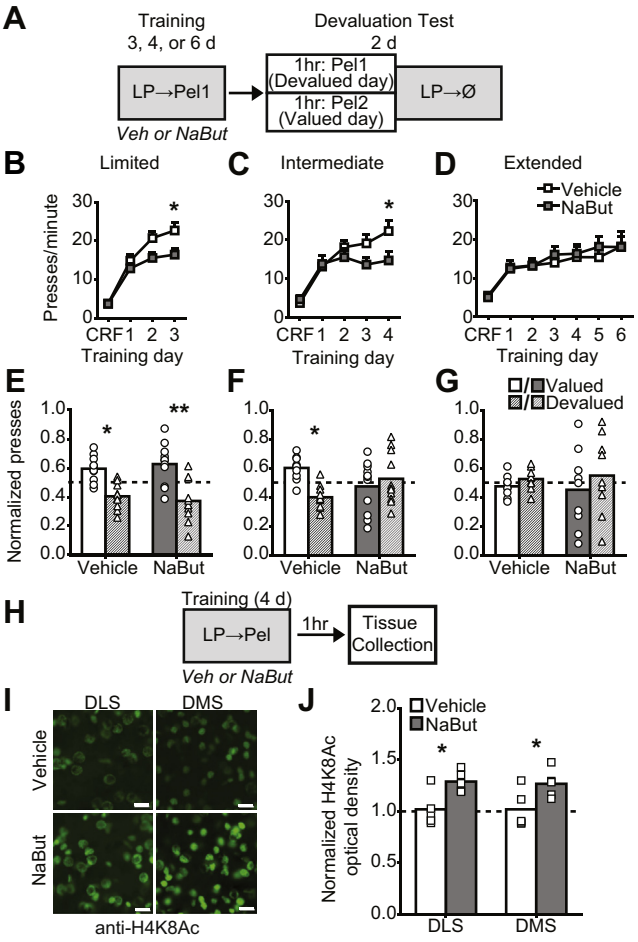


**Figure 1.** Effect of posttraining histone deacetylase inhibition on instrumental learning, behavioral strategy, and acetylation at histone H4. **(A)** Schematic representation of procedures. **(B–D)** Instrumental (lever press → reward) training performance for rats given **(B)** limited ($n = 11$ per group), **(C)** intermediate ($n = 11$–$12$ per group), or **(D)** extended ($n = 9$ per group) instrumental training (data presented as mean + SEM). On the first training day lever pressing was continuously reinforced (CRF) with food-pellet delivery. A random-interval 30-second reinforcement schedule was in place thereafter. **(E–G)** Lever presses during the subsequent devaluation tests normalized to total presses across both tests for the valued [valued state presses/(valued + devalued state presses)] and devalued [devalued state presses/(valued + devalued state presses)] states for rats that received **(E)** limited, **(F)** intermediate, or **(G)** extended training. Dashed line indicates point of equal responding between tests (data presented as mean + scatter). **(H)** Schematic representation of procedures. **(I, J)** Representative immunofluorescent image and quantification of acetylation of histone H4 lysine 8 (H4K8Ac) ($n = 4$–$6$ per condition) 1 hour following instrumental training/drug treatment. Scale bar = 20 μm. Data normalized to vehicle control (dashed line). $^*p < .05$; $^{**}p < .01$. d, days; DLS, dorsolateral striatum; DMS, dorsomedial striatum; LP, lever press; NaBut, sodium butyrate; Pel, pellet; Veh, vehicle.

intermediate training, NaBut-treated animals became insensitive to devaluation of the earned reward, failing to reduce responding in the devalued versus valued state, while lever pressing in vehicle-treated subjects remained sensitive to devaluation (Figure 1F) (devaluation [$F_{1,20}$ = 1.68, $p$ = .21], drug [$F_{1,20}$ = 0.74, $p$ = .40], drug × devaluation [$F_{1,20}$ = 4.85, $p$ = .04]). In separate groups, we found that posttraining HDAC inhibition accelerated habit formation when behavioral strategy was assayed by reversal of the action-outcome contingency (Supplemental Figure S2), which does not involve a value or satiety manipulation, and occurred even when rats were trained on a random-ratio reinforcement schedule (Supplemental Figure S3) known to promote goal-directed control (1). Importantly, this effect did not manifest if NaBut was administered outside the memory-consolidation window (Supplemental Figure S4). These data reveal that inhibition of class I HDACs following instrumental conditioning facilitates the transition to habitual control of behavior, allowing habits to dominate at a point in training that behavior would normally remain primarily goal directed.

## Effect of Posttraining HDAC Inhibition on Dorsostriatal Histone Acetylation and Neuronal Activation

HDACs constrain histone acetylation levels by removing acetyl groups from histone tails, and HDAC inhibition can prevent the rapid deacetylation of histone proteins (19); therefore, HDAC inhibitor treatment should cause increased histone acetylation in brain regions in which HATs are activated (21). In a separate group of subjects, we next used immunofluorescence (26,31,32) to determine the brain region–specific effects of peripheral NaBut treatment following instrumental training (Figure 1H). We hypothesized that histone acetylation would be increased in the DLS, a region critical for habit formation (11) and demonstrated to be transcriptionally active during habit learning (17). We focused on histone H4 lysine 8 (H4K8Ac), a histone acetylation mark implicated in memory formation (33,34). One hour after the last intermediate training session and drug delivery, H4K8Ac was significantly higher in the DLS ($t_8$ = 3.08, $p$ = .02) of NaBut-treated subjects compared with control subjects (Figure 1I–J, see also Supplemental Figure S5). H4K8Ac was also elevated in the DMS ($t_8$ = 2.41, $p$ = .04), but there were no significant differences detected in any other regions examined (see Supplemental Table S1). This increase in histone acetylation was restricted to neurons in the DMS and occurred in both neuronal and nonneuronal cells in the DLS (Supplemental Figure S6). Using real-time quantitative polymerase chain reaction (qPCR), we found that both the DMS and DLS were engaged (as measured by immediately early gene expression) during instrumental conditioning and this was not significantly altered by posttraining HDAC inhibition (Supplemental Figure S7).

## Effect of Training and Posttraining HDAC Inhibition on HDAC3 Occupancy at Learning-Related Gene Promoters and Gene Expression in the DLS

By removing acetyl groups, HDACs typically repress gene transcription (19). That posttraining HDAC inhibition accelerated the transition to habit suggests that HDACs may normally be engaged early in learning to restrain gene expression supporting habit formation. If this is true, then HDAC occupancy at the promoters of key learning-related genes may be enriched with intermediate instrumental training to prevent dominance by the habit strategy, but removed back to baseline levels when conditions are ripe for habit to dominate, e.g., overtraining, or by HDAC inhibition. To test this, we used chromatin immunoprecipitation coupled with qPCR to examine HDAC occupancy at the promoter regions of *Bdnf1*, *Nr4a1*, and *Nr4a2*. These genes were selected because they have been shown to be regulated by histone acetylation and involved in long-term memory formation (30,31,33–35), and are regulated by cyclic adenosine monophosphate response element binding protein (CREB) (36–38), a transcription factor implicated in habit-like behaviors (13,16,17). We focused on the DLS, given its crucial role in habit formation and findings of training-related activity and NaBut regulation of H4K8Ac in this region, and HDAC3, the most highly expressed class I HDAC in the striatum (39).

One hour following the last intermediate training session (Figure 2A), HDAC3 occupancy at the *Bdnf* exon 1 promoter was enriched relative to home-cage control animals ($p < .001$), but returned to control levels with extended training and was not elevated above control levels when intermediate training

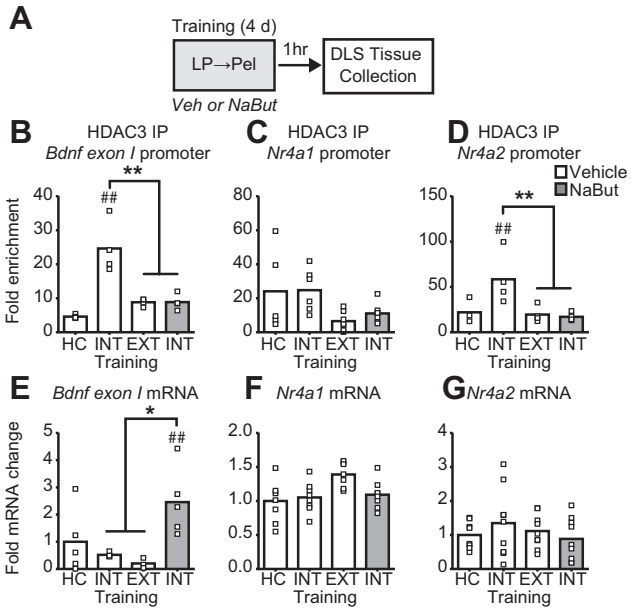


**Figure 2.** Effect of training and posttraining histone deacetylase (HDAC) inhibition on HDAC3 occupancy at learning-related gene promoters and gene expression in the dorsolateral striatum (DLS). **(A)** Schematic representation of procedures. **(B–D)** Chromatin immunoprecipitation was performed with anti-HDAC3 followed by quantitative polymerase chain reaction to identify HDAC3 binding to the **(B)** *Bdnf1*, **(C)** *Nr4a1*, or **(D)** *Nr4a2* promoters in the DLS of home-cage (HC) control subjects or following either intermediate (INT) or extended (EXT) training in vehicle-treated rats, or sodium butyrate (NaBut) treatment post–intermediate training. Data presented as fold change relative to immunoglobulin G (% input/immunoglobulin G). **(E–G)** Messenger RNA (mRNA) expression of **(E)** *Bdnf1*, **(F)** *Nr4a1*, and **(G)** *Nr4a2* in the DLS. $^{*}p < .05$, $^{**}p < .01$, between groups; $^{\#\#}p < .01$ relative to HC group. d, days; IP, immunoprecipitation; LP, lever press; Pel, pellet; Veh, vehicle.

was followed by NaBut treatment (Figure 2B) ($F_{3,12} = 18.53$, $p < .001$). A similar pattern was detected for HDAC3 occupancy at the promoter for *Nr4a2* (Figure 2D) ($F_{3,12} = 5.74$, $p = .01$). Although there was an overall effect of training/treatment group on HDAC3 occupancy at the *Nr4a1* promoter (Figure 2C) ($F_{3,21} = 3.12$, $p = .05$), in no case was HDAC3 enrichment at this promoter significantly different from the control group ($p > .05$). Expression of *Bdnf1* (Figure 2E), quantified with qPCR, was significantly elevated 1 hour following intermediate training with NaBut treatment relative to home-cage control subjects ($t_{13} = 2.41$, $p = .03$), the intermediate trained control group ($t_{13} = 3.02$, $p = .01$), and control subjects following extended training ($t_{13} = 3.23$, $p = .01$). A similar pattern was detected in *Bdnf9* expression (Supplemental Figure S8). *Nr4a1* and *Nr4a2* expression (Figure 2F, G) was not significantly different from the control subjects in any condition.

These data suggest that HDAC3 occupancy at the *Bdnf1* and *Nr4a2* promoters in the DLS is normally enriched during early instrumental learning, but becomes dissociated from these gene promoters as habits dominate behavioral control. Posttraining HDAC inhibition accelerates both habit formation and removal of HDAC3 from the promoters of these key learning genes, and also induces *Bdnf* expression. Therefore, HDAC inhibition immediately following instrumental training caused a transcriptionally permissive, hyperacetylated histone state, disengaged HDAC3 from specific gene promoters in the DLS, and facilitated behavioral control by the habit system.

## Effect of HDAC3 Manipulation in DLS on Habit Formation

That HDAC3 is engaged in the DLS during early learning and then disengaged with the overtraining that promotes habit, suggests that decreasing DLS HDAC3 activity may promote habit formation. We tested this hypothesis in two ways: first, by pharmacologically inhibiting HDAC3 activity specifically in the DLS immediately after each instrumental training session; and, second, by expressing a dominant negative HDAC3 point mutant (34) in the DLS before all training to selectively disrupt HDAC3 enzymatic activity, without affecting protein-protein interactions (40,41) (Figure 3A). In both cases, habit formation was potentiated, recapitulating the systemic HDAC inhibition result. Posttraining intra-DLS infusion of the selective HDAC3 inhibitor RGFP966 (1.0 ng × 0.5 μL × side) (31,42,43) elevated H4K8Ac in the DLS (Figure 3B, C; see also Supplemental Figure S9) ($t_8 = 3.15$, $p = .014$), but not the adjacent DMS (Figure 3B) (normalized H4K8Ac optical density: vehicle, 1.011 ± 0.10; RGFP966, 1.00 ± 0.27 [$t_3 = 0.06$, $p = .954$]) compared with vehicle-treated control subjects. This treatment did not alter the acquisition of instrumental lever-press behavior (Figure 3D) (training day [$F_{4,68} = 32.61$, $p < .001$], drug [$F_{1,17} = 0.95$, $p = .34$], drug × day [$F_{4,68} = 2.32$, $p = .07$]), but did render this behavior insensitive to devaluation of the earned reward under conditions in which intermediate-trained control subjects remained sensitive (Figure 3E) (devaluation [$F_{1,17} = 10.83$, $p = .004$], drug [$F_{1,17} = 1.12$, $p = .31$], drug × devaluation [$F_{1,17} = 7.25$, $p = .02$]). Similarly, expressing a dominant negative point mutant of HDAC3 (AAV2/1-CMV-HDAC3Y298H-V5) in the DLS produced H4K8 hyperacetylation (Figure 3F, G) ($t_{10} = 3.91$, $p = .003$) and insensitivity to outcome devaluation (Figure 3I) (devaluation [$F_{1,14} = 3.74$ $p = .07$], virus [$F_{1,14} = 0.47$, $p = .506$], virus × devaluation [$F_{1,17} = 6.19$, $p = .03$]), without altering lever-pressing acquisition (Figure 3H) (training day [$F_{4,56} = 29.21$, $p < .001$], virus [$F_{1,14} < 0.01$, $p = .99$], virus × day [$F_{4,56} = 0.56$, $p = .69$]). These results demonstrate that attenuating DLS HDAC3 activity promotes habit formation.

The combined data strongly suggest that in the DLS HDAC3 may normally be engaged to restrain habits when sufficient action repetition has not yet occurred. If this is true, then increasing HDAC3 activity in the DLS should slow or prevent habit formation. To test this, we overexpressed HDAC3 in the DLS (AAV2/1-CMV-HDAC3-V5), used immunofluorescence to confirm that this reduced H4K8Ac in the DLS (Figure 3J, K) ($t_9 = 2.29$, $p = .048$), and gave subjects extended instrumental training known to promote habit. All subjects similarly acquired the lever-press behavior (Figure 3L) (training day [$F_{6,126} = 81.44$, $p < .001$], virus [$F_{1,21} = 0.06$, $p = .81$], virus × day [$F_{6,126} = 0.44$, $p = .85$]). As expected, control subjects showed evidence of habits (insensitivity to devaluation), whereas subjects with HDAC3 overexpressed in the DLS were unable to form behavioral habits and continued to show sensitivity to devaluation (Figure 3M) (devaluation [$F_{1,21} = 2.24$, $p = .15$], virus [$F_{1,21} = -8.17$, $p > .999$], virus × devaluation [$F_{1,21} = 6.45$, $p = .02$]), even after extensive overtraining (Supplemental Figure S10). Together, these data demonstrate that in the DLS HDAC3 is a critical negative regulator of habit formation.

## Effect of HDAC3 Manipulation in DMS on Habit Formation

We found that systemic post–instrumental-training HDAC inhibition increased H4K8Ac in not only the DLS, but also the DMS. To further understand how altered regulation of gene transcription in these dissociable brain systems contributes to a transition in behavioral control, we next asked whether manipulating HDAC3 in the DMS would modify the progression of instrumental strategy (Figure 4A). Both posttraining intra-DMS HDAC3 inhibition (RGFP966, 1.0 ng × 0.5μL × side; DMS [$t_7 = 2.54$, $p = .04$], DLS [vehicle, 1.00 ± 0.02 normalized H4K8Ac optical density versus RGFP966 1.09 ± 0.07; $t_7 = 1.30$, $p = .23$]) (Figure 4B, C; see also Supplemental Figure S11) and expression of the dominant negative HDAC3 point mutant in the DMS (Figure 4F, G) ($t_7 = 4.32$, $p = .004$) induced a hyperacetylated H4K8 state that was restricted to the DMS. Neither treatment affected acquisition of the lever-press behavior (RGFP966: Figure 4D; HDAC3 point mutant: Figure 4H) (RGFP966: training day [$F_{4,64} = 45.38$, $p < .001$], drug [$F_{1,16} = 0.06$, $p = .81$], drug × day [$F_{4,64} = 0.12$, $p = .98$]; HDAC3 point mutant: day [$F_{4,84} = 32.38$, $p < .001$], virus [$F_{1,21} = 0.19$ $p = .67$], virus × day [$F_{4,84} = 0.44$, $p = .78$]). To our surprise, given the canonical function of the DMS in goal-directed, not habit, learning (7,8), both posttraining intra-DMS RGFP966 (Figure 4E) (devaluation [$F_{1,16} = 1.16$ $p = .298$], drug [$F_{1,16} = 0.00$, $p > .999$], drug × devaluation [$F_{1,16} = 4.79$, $p = .04$]) and DMS HDAC3 point mutant expression (Figure 4I) (devaluation [$F_{1,21} = 1.94$, $p = .18$], virus [$F_{1,21} = 17.61$, $p < .001$], virus × devaluation [$F_{1,21} = 5.52$, $p = .03$]) potentiated habit formation, as indicated by insensitivity to devaluation of the earned reward following intermediate training.

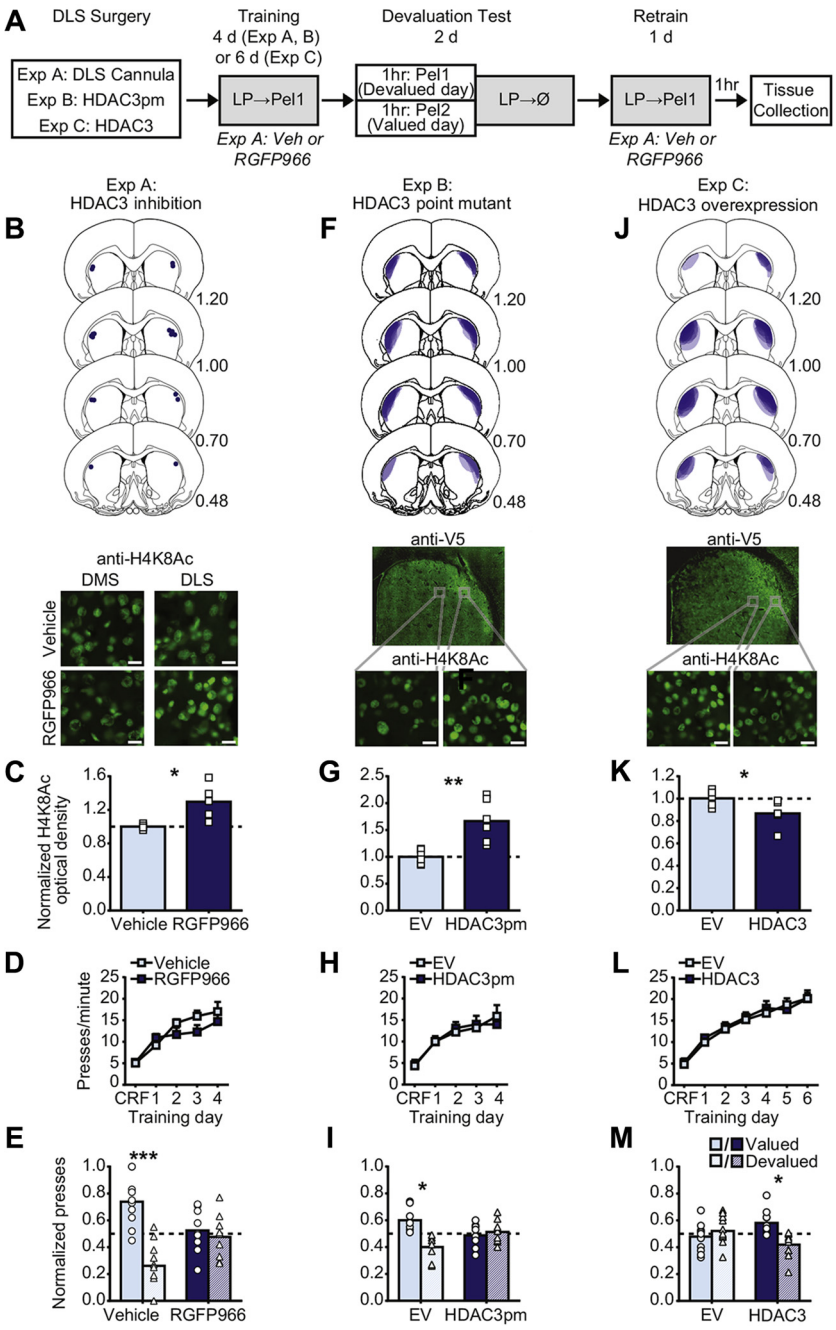


**Figure 3.** Effect of histone deacetylase 3 (HDAC3) manipulation in dorsolateral striatum (DLS) on habit formation. **(A)** Schematic representation of procedures. **(B)** Top panel: schematic representation of injector tips in the DLS. Numbers to the lower right of each section represent distance (mm) anterior to bregma. Coronal section drawings taken from Paxinos and Watson (96). Bottom panel: representative immunofluorescent images of acetylation of histone H4 lysine 8 (H4K8Ac) in the dorsomedial striatum (DMS) (left) and DLS (right) 1 hour following instrumental training/intra-DLS vehicle (top) or RGFP966 (bottom) infusion. Scale bar = 20 μm. **(F, J)** Top panel: schematic representation of **(F)** HDAC3 point mutant (HDAC3pm) or **(J)** HDAC3 expression in the DLS for all subjects. Middle panel: representative immunofluorescent images of V5-tagged **(F)** HDAC3pm or **(J)** HDAC3 expression in the DLS. Bottom panel: representative immunofluorescent images of H4K8Ac in rats expressing the **(F)** HDAC3pm or **(J)** HDAC3 either outside (left) or inside (right) of the expression zone. **(C, G, K)** Quantification of H4K8Ac for rats receiving **(C)** intra-DLS vehicle or RGFP966 infusion ($n$ = 5/group), **(G)** intra-DLS empty vector (EV) or HDAC3pm ($n$ = 6 per group), or **(K)** intra-DLS empty vector or HDAC3 overexpression ($n$ = 5-6 per group; data presented as mean + scatter). **(D, H, L)** Instrumental training performance for rats given posttraining **(D)** intra-DLS vehicle or RGFP966 infusions ($n$ = 10–12 per group), **(H)** intra-DLS empty vector or HDAC3pm ($n$ = 8/group), or **(L)** intra-DLS EV or HDAC3 ($n$ = 11–13 per group; data presented as mean + SEM). **(E, I, M)** Normalized lever presses during the subsequent devaluation tests for rats given posttraining **(E)** intra-DLS vehicle or RGFP966 infusions, **(I)** intra-DLS EV or HDAC3pm, or **(M)** intra-DLS EV or HDAC3. $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$. CRF, continuous reinforcement; d, days; Exp, experiment; LP, lever press; Pel, pellet; Veh, vehicle.

Conversely, DMS HDAC3 overexpression reduced H4K8Ac (Figure 4J, K) ($t_8$ = 3.27, $p$ = .01), did not alter lever-pressing acquisition (Figure 3L) (day [$F_{6,132}$ = 61.69, $p < .001$], virus [$F_{1,22}$ = 1.36, $p$ = .26], virus × day [$F_{6,132}$ = 2.01, $p$ = .07]), but prevented habit formation (Figure 4M) (devaluation [$F_{1,22}$ = 7.06, $p$ = .01], virus [$F_{1,22}$ = 2.68, $p$ = .12], virus × devaluation [$F_{1,22}$ = 4.34, $p$ = .049]), even with extensive overtraining (Supplemental Figure S12). These unexpected results demonstrate that, as in in the DLS, DMS HDAC3 negatively regulates the transition to habit.

Given this surprising finding, we next examined normal HDAC3 engagement in the DMS with instrumental training. Using chromatin immunoprecipitation coupled with qPCR (Figure 5A), we found that, unlike in the DLS, HDAC3 occupancy at *Bdnf1* was not significantly altered 1 hour following instrumental training or intermediate training followed by systemic NaBut treatment (Figure 5B) ($F_{3,22}$ = 1.87, $p$ = .16). Similarly, in no case was HDAC3 enrichment at the *Nr4a2* promoter significantly different from the control subjects (Figure 5D) ($F_{3,21}$ = 2.74, $p$ = .07). Instead, HDAC3 occupancy

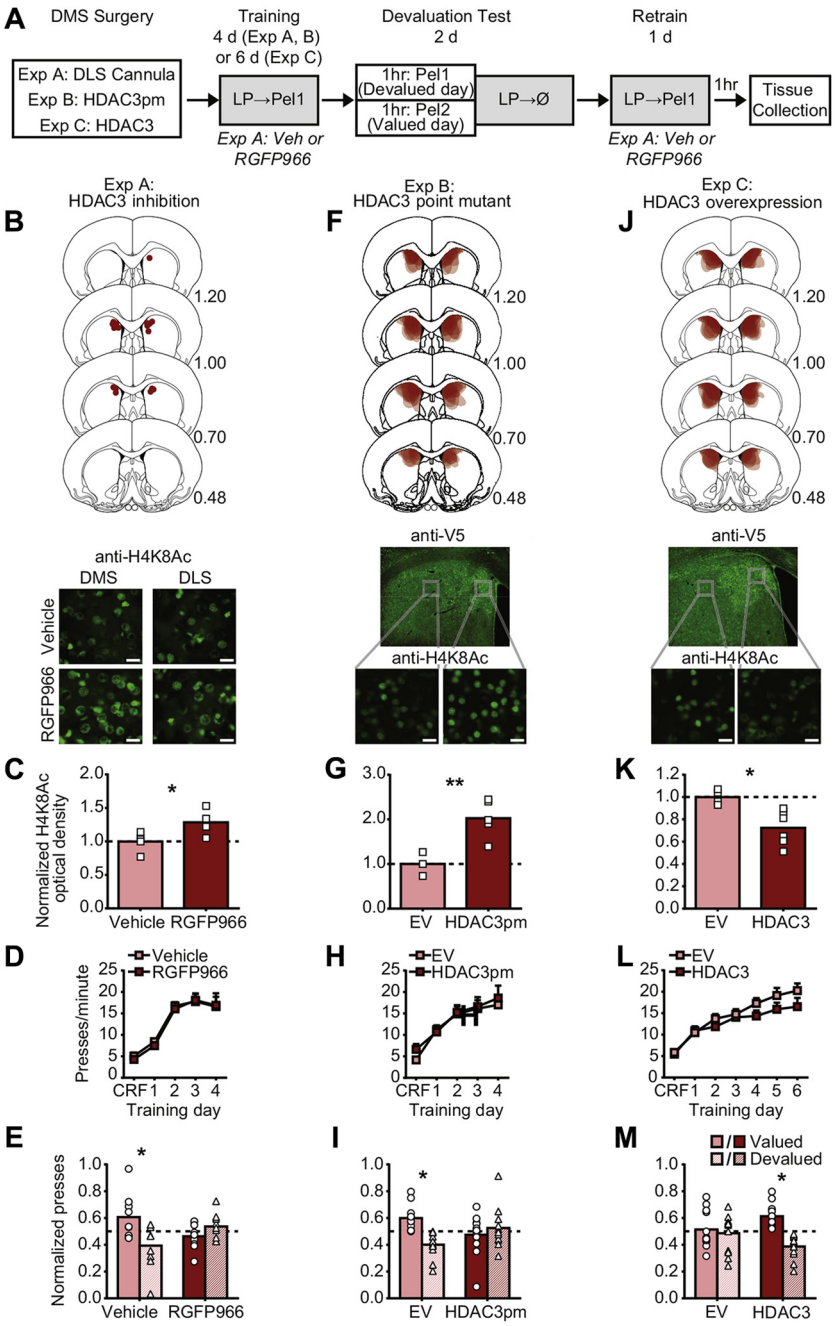


**Figure 4.** Effect of histone deacetylase 3 (HDAC3) manipulation in the dorsomedial striatum (DMS) on habit formation. **(A)** Schematic representation of procedures. **(B)** Top panel: schematic representation of injector tips in the DMS. Numbers to the lower right of each section represent distance (mm) anterior to bregma. Bottom panel: representative immunofluorescent images of acetylation of histone H4 lysine 8 (H4K8Ac) in the DMS (left) and dorsolateral striatum (DLS) (right) 1 hour following instrumental training/intra-DMS (top) vehicle or RGFP966 (bottom) infusion. Scale bar = 20 μm. **(F, J)** Top panel: schematic representation of **(F)** HDAC3 point mutant (HDAC3pm) or **(J)** HDAC3 expression in the DMS for all subjects. Middle panel: representative immunofluorescent images of **(F)** HDAC3pm or **(J)** HDAC3 expression in the DMS. Bottom panel: representative immunofluorescent images of H4K8Ac in rats expressing the **(F)** HDAC3pm or **(J)** HDAC3 either outside (left) or inside (right) of the expression zone. **(C, G, K)** Quantification of H4K8Ac for rats receiving **(C)** intra-DMS vehicle or RGFP966 infusion ($n$ = 4–5 per group), **(G)** intra-DMS empty vector (EV) or HDAC3pm ($n$ = 4–5 per group), or **(K)** intra-DMS EV or HDAC3 overexpression ($n$ = 4–6 per group; data presented as mean + scatter). **(D, H, L)** Instrumental training performance for rats given posttraining **(D)** intra-DMS vehicle or RGFP966 infusions ($n$ = 9 per group), **(H)** intra-DMS EV or HDAC3pm ($n$ = 11–12 per group), or **(L)** intra-DMS EV or HDAC3 ($n$ = 12 per group; data presented as mean + SEM). **(E, I, M)** Normalized lever presses during the subsequent devaluation tests for rats given posttraining **(E)** intra-DMS vehicle or RGFP966 infusions, **(I)** intra-DMS EV or HDAC3pm, or **(M)** intra-DMS EV or HDAC3. *$p$ < .05; **$p$ < .01. CRF, continuous reinforcement; d, days; Exp, experiment; LP, lever press; Pel, pellet; Veh, vehicle.

at the *Nr4a1* promoter (Figure 5C) ($F_{3,12}$ = 7.76, $p$ = .004) was increased relative to home-cage control subjects with intermediate training ($p$ < .05) and returned to control levels following extended training, and was not elevated following intermediate training with systemic NaBut treatment. Follow-up messenger RNA analyses (Figure 5E–G) revealed that *Nr4a2* expression (Figure 5G) ($F_{3,31}$ = 3.45, $p$ = .03) was attenuated relative to home-cage control subjects with intermediate training ($p$ < .01) and intermediate training with systemic NaBut treatment ($p$ < .05). *Bdnf1* and *Nr4a1* were not significantly altered (Figure 5E; see also Supplemental Figure S8) (*Bdnf1* [$F_{3,16}$ = 1.57, $p$ = .24], *Nr4a1* [$F_{3,25}$ = 1.87, $p$ = .55]). These results suggest that HDAC3 activity in the DMS and DLS is differentially regulated by instrumental training as habits form.

## DISCUSSION

Reward seeking and decision making are controlled by a balance between two systems, one reflective, involving prospective

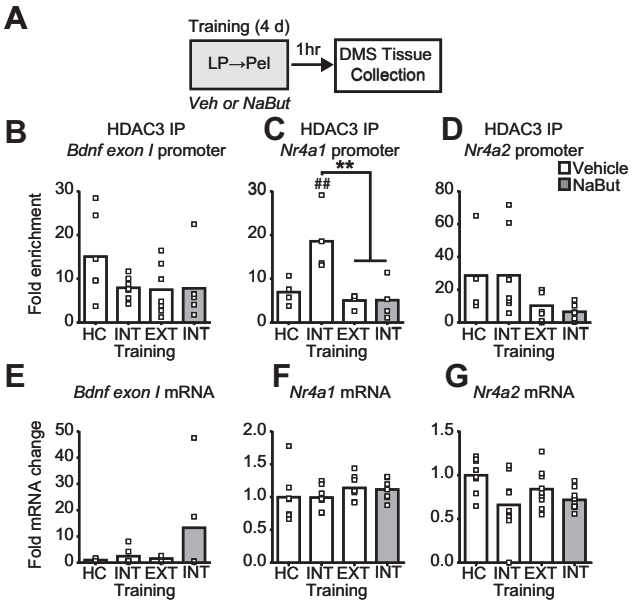


**Figure 5.** Effect of training and posttraining histone deacetylase (HDAC) inhibition on HDAC3 occupancy at learning-related gene promoters and gene expression in the dorsomedial striatum (DMS). **(A)** Schematic representation of procedures. **(B–D)** Chromatin immunoprecipitation was performed with anti-HDAC3 followed by quantitative polymerase chain reaction to identify **(B)** HDAC3 binding to the *Bdnf1*, **(C)** *Nr4a1*, or **(D)** *Nr4a2* promoters in the DMS of home-cage (HC) control subjects or following either intermediate (INT) or extended (EXT) training in vehicle-treated rats, or sodium butyrate (NaBut) treatment post–intermediate training. **(E–G)** Data presented as fold change relative to immunoglobulin G (% input/immunoglobulin G) messenger RNA (mRNA) expression of **(E)** *Bdnf1*, **(F)** *Nr4a1*, and **(G)** *Nr4a2* in the DMS. $^{**}p < .01$, between groups; $^{\#\#}p < .01$ relative to HC control group. d, days; IP, immunoprecipitation; LP, lever press; Pel, pellet; Veh, vehicle.

consideration of learned action consequences, and one reflexive, allowing common behaviors to be automatically triggered by antecedent events on the basis of their past success. The data here provide converging evidence in support of dorsostriatal HDAC3 as a critical molecular regulator of the transition of behavioral control to the habit strategy. Systemic HDAC inhibition during instrumental acquisition increases histone acetylation in the dorsal striatum and accelerates habitual control of behavior. HDAC3 occupancy at specific learning-related gene promoters in the dorsal striatum is reduced, relative to early training, when habits form with overtraining, and this is mimicked by HDAC inhibition. Using local pharmacological and viral manipulations, HDAC3 activity was found to constrain habit learning in the DLS, previously implicated in habit (11), and also in the DMS, which has been canonically ascribed a role in the opposing, goal-directed, strategy (7,8).

HDAC3 in the dorsal striatum functions as a negative regulator of habit, as evidenced by its disruption accelerating the rate at which behavioral control transitioned to dominance by habit, and its overexpression in the dorsal striatum preventing subjects from forming habits under conditions that would normally promote them to do so. The temporally restricted effect of posttraining HDAC inhibition suggests that HDAC3 negatively regulates the consolidation of habit memories. Habits are slow to form, being gradually acquired with repetition of successful actions in the presence of consistent stimuli, but once fully formed habits can be executed almost automatically, freeing attention to be focused elsewhere (1,2). The current data suggest that in the dorsal striatum the repressive enzyme HDAC3 could be a molecular "brake" (22,23) on this type of learning, being engaged to slow habit formation and preserve behavioral control by the more cognitively taxing, but less error-prone, goal-directed system until enough successful repetition has proceeded to ensure sufficient accuracy of the habit. In support of this, early in training, when habits do not yet dominate behavioral control, HDAC3 occupancy at the *Bdnf1* and *Nr4a2* promoters in the DLS and at the *Nr4a1* promoter in the DMS is enriched and then returns to baseline levels with the repeated training that promotes habit. That overexpression of HDAC3 in the dorsal striatum prevented habit formation further suggests that, under suitable conditions (e.g., repeated success), an instrumental learning opportunity triggers activity-dependent signaling that removes HDAC3 to create the transcriptionally permissive state that allow habits to strengthen and, eventually, come to control behavior. Dorsal striatal HDAC3, therefore, normally curtails habit.

Consolidation of long-term memories, such as habits, depends on gene transcription (44). HDAC3 may, therefore, function to curtail the gene transcription underlying habit. Transcription regulated by CREB has been shown to be essential for long-term memory (45–48), and CREB function in the dorsal striatum is crucial for habit learning (13). The enhancement of memory by HDAC inhibition has been demonstrated to occur through regulation of CREB–regulated genes (33,36), including *Bdnf*, *Nr4a1*, and *Nr4a2* (36,49), genes themselves implicated in learning (33,35–37,50–52). Here we found that as habits formed, HDAC3 was disengaged (relative to its enriched state during early learning) from the *Bdnf1* and *Nr4a2* promoters in the DLS and the *Nr4a1* promoter in the DMS. HDAC inhibition, which accelerated habit formation, similarly altered HDAC3 occupancy at these promoters, and, in some cases, induced the expression of these genes. These data suggest that a probable mechanism for striatal HDAC3 moderation of habit formation is via regulation of CREB–regulated genes. HDAC3 represses transcription by removing acetylation and recruiting complementary repressive enzymes, such as methyltransferases or phosphatases. HDAC removal allows HATs to promote histone acetylation, which decreases affinity of histone tails for DNA and serves as a recruitment signal for transcriptional coactivators (19) that can promote active gene expression subserving synaptic plasticity and learning (22–24). Although the data show that H4K8Ac is increased in the dorsal striatum by posttraining HDAC inhibition, the precise mechanisms of HDAC3 regulation of CREB–regulated genes in the dorsal striatum, e.g., whether it is via acetylation at H4K8, other histone sites, a combination of marks, and/or through corepressor mechanisms, remains to be explored.

Interestingly, HDAC3 binding at gene promoters and gene expression did not always perfectly match. This discrepancy could be due to differential temporal dynamics between transcriptional activation and HDAC3-promoter interactions (34), but it also likely indicates that HDAC3 binding is not the sole

determinant of expression of the genes examined here. Indeed, gene expression is regulated by a complex concert of factors, including repressive enzymes, such as HDAC3, and activating enzymes, such as transcription factors and HATs. Recruitment of HATs, such as CREB (28,36), are required for HDAC3 regulation of gene transcription and memory. Moreover, it is also possible that the nonspecific class I HDAC inhibition treatment engaged repressive mechanisms (53) additional to those normally engaged to regulate gene expression by learning alone, perhaps suggesting alternative, nonnatural routes to habit. Future experiments assessing other transcriptional regulators and/or using genome-wide approaches are needed to further elucidate the molecular mechanisms supporting habit.

Surprisingly, HDAC3 was found to negatively regulate habit in both the DLS and DMS. HDAC3 inhibition restricted to the DMS potentiated habit formation, whereas DMS-specific HDAC3 overexpression, while not affecting instrumental learning per se, prevented behavioral control from transitioning to dominance by the habit system, completely recapitulating the effect found with identical DLS manipulations. The overexpression result, along with data demonstrating HDAC3 enrichment early in training and removal back to baseline with overtraining at the *Nr4a1* promoter in the DMS, provides evidence that DMS HDAC3 activity functions normally to repress habit. These results were unexpected in light of the canonical view that the DMS is crucial for action-outcome learning (54,55) and evidence that DMS lesions force behavioral control by the habit system (7–9). Potential functional differences between the anterior and posterior DMS may help to understand these surprising results. The anterior DMS was targeted here based on the locus of systemic HDAC inhibition–induced increase in histone acetylation, whereas seminal lesion results implicated the posterior DMS in goal-directed learning (8). More recent work, however, has demonstrated that both the anterior and posterior DMS are crucial for the action-outcome learning underlying goal-directed control (9). Previous data suggest that DLS circuits might store habit-related information (56–59), with memories vital for goal-directed control stored in the DMS (60–62). One possibility is that HDAC3 may regulate the formation and storage of habit memories in the DLS, while in the DMS it depotentiates the action-outcome memories underlying goal-directed control as deliberation becomes less required and actions become chunked (63,64) into stereotyped units. Indeed, neurons in the DLS will show chunking-related activity that strengthens with training, while simultaneously DMS neurons show activity at deliberation points that wanes with extended training (65). It is also possible that HDAC3 regulates DMS indirect-pathway projections, activity that has been shown to promote habit (66). In either case, the distinct HDAC3 occupancy patterns at learning-related gene promoters in the DLS versus DMS suggest differential transcriptional regulation by HDAC3 in these subregions. Importantly, these results challenge the strict dissociation between DMS and DLS function in goal-directed and habitual control of behavior.

These data reveal a new molecular directive of habit formation. Striatal HDAC3 functions as a molecular brake over habit, being engaged to slow the transition to habit and removed when the conditions are ripe for habits to dominate. Epigenetic dysfunction has been implicated in the etiology of many psychiatric disease states (67–71). The balance between goal-directed and habitual control is also disrupted in these conditions. Indeed, deficits in the acquisition and execution of behavioral habits are symptoms of both Huntington's disease (72) and Parkinson's disease (73–75). An overreliance on habit has been associated with the compulsivity that manifests across a range of psychiatric diseases (6,76), including obsessive-compulsive disorder (77,78), autism spectrum disorder (79), schizophrenia (80,81), addiction (82), alcoholism (83,84), and compulsive overeating (85). Moreover, stress, a predisposing condition to many psychiatric illnesses, can lead to abnormal HDAC activity (68,86), which could, given the current result, lead to its ability to potentiate habits (87–89) and thereby promote maladaptive compulsive behavior (90). The current data, therefore, suggest a potential molecular mechanism for such maladaptive behavior (90) and support notions of HDAC3 as a promising target for therapeutic intervention (23,67,91–95). These data also highlight the potential for unintended effects on habit learning by HDAC3 therapeutic manipulations.

## ACKNOWLEDGMENTS AND DISCLOSURES

This research was supported by a Hellman Foundation Fellowship, UCLA Faculty Career Development award, National Institutes of Health Grant Nos. DA035443 (to KMW), DA025922 (to MAW), and AG051807 (to MAW), and startup funds from the UCLA Life Sciences Division (to PJK).

MM and KMW designed the research and analyzed and interpreted the data, with assistance from MAW and PJK. MM conducted the research with assistance from VYG and MDM. DPM and MAW prepared and contributed viral constructs and conducted chromatin immunoprecipitation experiments. MM, NAA, and PJK collected and analyzed real-time quantitative polymerase chain reaction data. MM and KMW wrote the manuscript with assistance from MAW and PJK.

The authors report no biomedical financial interests or potential conflicts of interest.

## ARTICLE INFORMATION

From the Department of Psychology (MM, VYG, NAA, MDM, PJK, KMW) and Brain Research Institute (PJK, KMW), University of California, Los Angeles, Los Angeles; and the Department of Neurobiology and Behavior (DPM, MAW), University of California, Irvine, Irvine, California.

Address correspondence to Kate M. Wassum, Ph.D., Department of Psychology, University of California, Los Angeles, 1285 Franz Hall, Box 951563, Los Angeles, CA 90095-1563; E-mail: kwassum@ucla.edu.

Received Jul 12, 2017; revised Jan 26, 2018; accepted Jan 29, 2018.

Supplementary material cited in this article is available online at https://doi.org/10.1016/j.biopsych.2018.01.025.

## REFERENCES

1. Dickinson A (1985): Actions and habits: The development of behavioural autonomy. Philos Trans R Soc Lond B 308:67–78.
2. Dolan RJ, Dayan P (2013): Goals and habits in the brain. Neuron 80:312–325.
3. Adams CD, Dickinson A (1981): Instrumental responding following reinforcer devaluation. Q J Exp Psychol 33:109–121.
4. Adams CD (1982): Variations in the sensitivity of instrumental responding to reinforcer devaluation. Q J Exp Psychol 34:77–98.
5. Gillan CM, Kosinski M, Whelan R, Phelps EA, Daw ND (2016): Characterizing a psychiatric symptom dimension related to deficits in goal-directed control. Elife 5:11305.

6. Voon V, Derbyshire K, Rück C, Irvine MA, Worbe Y, Enander J, *et al.* (2015): Disorders of compulsivity: A common bias towards learning habits. Mol Psychiatry 20:345–352.
7. Yin HH, Knowlton BJ, Balleine BW (2005): Blockade of NMDA receptors in the dorsomedial striatum prevents action-outcome learning in instrumental conditioning. Eur J Neurosci 22:505–512.
8. Yin HH, Ostlund SB, Knowlton BJ, Balleine BW (2005): The role of the dorsomedial striatum in instrumental conditioning. Eur J Neurosci 22:513–523.
9. Corbit LH, Janak PH (2010): Posterior dorsomedial striatum is critical for both selective instrumental and Pavlovian reward learning. Eur J Neurosci 31:1312–1321.
10. Liljeholm M, Tricomi E, O'Doherty JP, Balleine BW (2011): Neural correlates of instrumental contingency learning: Differential effects of action-reward conjunction and disjunction. J Neurosci 31:2474–2480.
11. Yin HH, Knowlton BJ, Balleine BW (2004): Lesions of dorsolateral striatum preserve outcome expectancy but disrupt habit formation in instrumental learning. Eur J Neurosci 19:181–189.
12. Tricomi E, Balleine BW, O'Doherty JP (2009): A specific role for posterior dorsolateral striatum in human habit learning. Eur J Neurosci 29:2225–2232.
13. Pittenger C, Fasano S, Mazzocchi-Jones D, Dunnett SB, Kandel ER, Brambilla R (2006): Impaired bidirectional synaptic plasticity and procedural memory formation in striatum-specific cAMP response element-binding protein-deficient mice. J Neurosci 26:2808–2813.
14. Hernandez A, Tan C, Mettlach G, Pozo K, Plattner F, Bibb JA (2016): Cdk5 modulates long-term synaptic plasticity and motor learning in dorsolateral striatum. Scientific Reports 6:29812.
15. Shiflett MW, Brown RA, Balleine BW (2010): Acquisition and performance of goal-directed instrumental actions depends on ERK signaling in distinct regions of dorsal striatum in rats. J Neurosci 30:2951–2959.
16. Brightwell JJ, Smith CA, Neve RL, Colombo PJ (2008): Transfection of mutant CREB in the striatum, but not the hippocampus, impairs long-term memory for response learning. Neurobiol Learn Mem 89:27–35.
17. Colombo PJ, Brightwell JJ, Countryman RA (2003): Cognitive strategy-specific increases in phosphorylated cAMP response element-binding protein and c-Fos in the hippocampus and dorsal striatum. J Neurosci 23:3547–3554.
18. Hernandez PJ, Schiltz CA, Kelley AE (2006): Dynamic shifts in corticostriatal expression patterns of the immediate early genes Homer 1a and Zif268 during early and late phases of instrumental training. Learn Mem 13:599–608.
19. Kouzarides T (2007): Chromatin modifications and their function. Cell 128:693–705.
20. Day JJ, Sweatt JD (2011): Epigenetic mechanisms in cognition. Neuron 70:813–829.
21. Peixoto L, Abel T (2013): The role of histone acetylation in memory formation and cognitive impairments. Neuropsychopharmacology 38:62–76.
22. Vogel-Ciernia A, Wood MA (2012): Molecular brake pad hypothesis: Pulling off the brakes for emotional memory. Rev Neurosci 23:607–626.
23. McQuown SC, Wood MA (2011): HDAC3 and the molecular brake pad hypothesis. Neurobiol Learn Mem 96:27–34.
24. Borrelli E, Nestler EJ, Allis CD, Sassone-Corsi P (2008): Decoding the epigenetic language of neuronal plasticity. Neuron 60:961–974.
25. Kilgore M, Miller CA, Fass DM, Hennig KM, Haggarty SJ, Sweatt JD, *et al.* (2010): Inhibitors of class 1 histone deacetylases reverse contextual memory deficits in a mouse model of Alzheimer's disease. Neuropsychopharmacology 35:870–880.
26. Malvaez M, Sanchis-Segura C, Vo D, Lattal KM, Wood MA (2010): Modulation of chromatin modification facilitates extinction of cocaine-induced conditioned place preference. Biol Psychiatry 67:36–43.
27. Lattal KM, Barrett RM, Wood MA (2007): Systemic or intrahippocampal delivery of histone deacetylase inhibitors facilitates fear extinction. Behav Neurosci 121:1125–1131.
28. Stefanko DP, Barrett RM, Ly AR, Reolon GK, Wood MA (2009): Modulation of long-term memory for object recognition via HDAC inhibition. Proc Natl Acad Sci U S A 106:9447–9452.
29. Ploense KL, Kerstetter KA, Wade MA, Woodward NC, Maliniak D, Reyes M, *et al.* (2013): Exposure to histone deacetylase inhibitors during Pavlovian conditioning enhances subsequent cue-induced reinstatement of operant behavior. Behav Pharmacol 24:164–171.
30. Bredy TW, Wu H, Crego C, Zellhoefer J, Sun YE, Barad M (2007): Histone modifications around individual BDNF gene promoters in prefrontal cortex are associated with extinction of conditioned fear. Learn Mem 14:268–276.
31. Malvaez M, McQuown SC, Rogge GA, Astarabadi M, Jacques V, Carreiro S, *et al.* (2013): HDAC3-selective inhibitor enhances extinction of cocaine-seeking behavior in a persistent manner. Proc Natl Acad Sci U S A 110:2647–2652.
32. Malvaez M, Mhillaj E, Matheos DP, Palmery M, Wood MA (2011): CBP in the nucleus accumbens regulates cocaine-induced histone acetylation and is critical for cocaine-associated behaviors. J Neurosci 31:16941–16948.
33. McQuown SC, Barrett RM, Matheos DP, Post RJ, Rogge GA, Alenghat T, *et al.* (2011): HDAC3 is a critical negative regulator of long-term memory formation. J Neurosci 31:764–774.
34. Kwapis JL, Alaghband Y, López AJ, White AO, Campbell RR, Dang RT, *et al.* (2017): Context and auditory fear are differentially regulated by HDAC3 activity in the lateral and basal subnuclei of the amygdala. Neuropsychopharmacology 42:1284–1294.
35. Intlekofer KA, Berchtold NC, Malvaez M, Carlos AJ, McQuown SC, Cunningham MJ, *et al.* (2013): Exercise and sodium butyrate transform a subthreshold learning event into long-term memory via a brain-derived neurotrophic factor-dependent mechanism. Neuropsychopharmacology 38:2027–2034.
36. Vecsey CG, Hawk JD, Lattal KM, Stein JM, Fabian SA, Attner MA, *et al.* (2007): Histone deacetylase inhibitors enhance memory and synaptic plasticity via CREB: CBP-dependent transcriptional activation. J Neurosci 27:6128–6140.
37. McNulty SE, Barrett RM, Vogel-Ciernia A, Malvaez M, Hernandez N, Davatolhagh MF, *et al.* (2012): Differential roles for Nr4a1 and Nr4a2 in object location vs. object recognition long-term memory. Learn Mem 19:588–592.
38. Ou LC, Gean PW (2007): Transcriptional regulation of brain-derived neurotrophic factor in the amygdala during consolidation of fear memory. Mol Pharmacol 72:350–358.
39. Broide RS, Redwine JM, Aftahi N, Young W, Bloom FE, Winrow CJ (2007): Distribution of histone deacetylases 1-11 in the rat brain. J Mol Neurosci 31:47–58.
40. Lahm A, Paolini C, Pallaoro M, Nardi MC, Jones P, Neddermann P, *et al.* (2007): Unraveling the hidden catalytic activity of vertebrate class IIa histone deacetylases. Proc Natl Acad Sci U S A 104:17335–17340.
41. Sun Z, Feng D, Fang B, Mullican SE, You SH, Lim HW, *et al.* (2013): Deacetylase-independent function of HDAC3 in transcription and metabolism requires nuclear receptor corepressor. Mol Cell 52:769–782.
42. Rumbaugh G, Sillivan SE, Ozkan ED, Rojas CS, Hubbs CR, Aceti M, *et al.* (2015): Pharmacological selectivity within class I histone deacetylases predicts effects on synaptic function and memory rescue. Neuropsychopharmacology 40:2307–2316.
43. Bieszczad KM, Bechay K, Rusche JR, Jacques V, Kudugunti S, Miao W, *et al.* (2015): Histone deacetylase inhibition via RGFP966 releases the brakes on sensory cortical plasticity and the specificity of memory formation. J Neurosci 35:13124–13132.
44. Alberini CM (2009): Transcription factors in long-term memory and synaptic plasticity. Physiol Rev 89:121–145.
45. Bourtchuladze R, Frenguelli B, Blendy J, Cioffi D, Schutz G, Silva AJ (1994): Deficient long-term memory in mice with a targeted mutation of the cAMP-responsive element-binding protein. Cell 79:59–68.
46. Yin JC, Wallach JS, Del Vecchio M, Wilder EL, Zhou H, Quinn WG, *et al.* (1994): Induction of a dominant negative CREB transgene specifically blocks long-term memory in Drosophila. Cell 79:49–58.
47. Guzowski JF, McGaugh JL (1997): Antisense oligodeoxynucleotide-mediated disruption of hippocampal cAMP response element binding protein levels impairs consolidation of memory for water maze training. Proc Natl Acad Sci U S A 94:2693–2698.

48. Pittenger C, Huang YY, Paletzki RF, Bourtchouladze R, Scanlin H, Vronskaya S, *et al.* (2002): Reversible inhibition of CREB/ATF transcription factors in region CA1 of the dorsal hippocampus disrupts hippocampus-dependent spatial memory. Neuron 34:447–462.
49. Tao X, Finkbeiner S, Arnold DB, Shaywitz AJ, Greenberg ME (1998): Ca2+ influx regulates BDNF transcription by a CREB family transcription factor-dependent mechanism. Neuron 20:709–726.
50. Lu Y, Christian K, Lu B (2008): BDNF: a key regulator for protein synthesis-dependent LTP and long-term memory? Neurobiol Learn Mem 89:312–323.
51. White AO, Kramár EA, López AJ, Kwapis JL, Doan J, Saldana D, *et al.* (2016): BDNF rescues BAF53b-dependent synaptic plasticity and cocaine-associated memory in the nucleus accumbens. Nat Commun 7:11725.
52. Gottmann K, Mittmann T, Lessmann V (2009): BDNF signaling in the formation, maturation and plasticity of glutamatergic and GABAergic synapses. Exp Brain Res 199:203–234.
53. Kennedy PJ, Feng J, Robison AJ, Maze I, Badimon A, Mouzon E, *et al.* (2013): Class I HDAC inhibition blocks cocaine-induced plasticity by targeted changes in histone methylation. Nat Neurosci 16:434–440.
54. Balleine BW, Delgado MR, Hikosaka O (2007): The role of the dorsal striatum in reward and decision-making. J Neurosci 27:8161–8165.
55. Balleine BW, O'Doherty JP (2010): Human and rodent homologies in action control: Corticostriatal determinants of goal-directed and habitual action. Neuropsychopharmacology 35:48–69.
56. O'Hare JK, Ade KK, Sukharnikova T, Van Hooser SD, Palmeri ML, Yin HH, *et al.* (2016): Pathway-specific striatal substrates for habitual behavior. Neuron 89:472–479.
57. Shan Q, Christie MJ, Balleine BW (2015): Plasticity in striatopallidal projection neurons mediates the acquisition of habitual actions. Eur J Neurosci 42:2097–2104.
58. Smith KS, Graybiel AM (2013): A dual operator view of habitual behavior reflecting cortical and striatal dynamics. Neuron 79:361–374.
59. Rueda-Orozco PE, Robbe D (2015): The striatum multiplexes contextual and kinematic information to constrain motor habits execution. Nat Neurosci 18:453–460.
60. Shan Q, Ge M, Christie MJ, Balleine BW (2014): The acquisition of goal-directed actions generates opposing plasticity in direct and indirect pathways in dorsomedial striatum. J Neurosci 34:9196–9201.
61. Gremel CM, Costa RM (2013): Orbitofrontal and striatal circuits dynamically encode the shift between goal-directed and habitual actions. Nat Commun 4:2264.
62. Gremel CM, Chancey JH, Atwood BK, Luo G, Neve R, Ramakrishnan C, *et al.* (2016): Endocannabinoid modulation of orbitostriatal circuits gates habit formation. Neuron 90:1312–1324.
63. Smith KS, Graybiel AM (2016): Habit formation. Dialogues Clin Neurosci 18:33–43.
64. Graybiel AM (1998): The basal ganglia and chunking of action repertoires. Neurobiol Learn Mem 70:119–136.
65. Thorn CA, Atallah H, Howe M, Graybiel AM (2010): Differential dynamics of activity changes in dorsolateral and dorsomedial striatal loops during learning. Neuron 66:781–795.
66. Li Y, He Y, Chen M, Pu Z, Chen L, Li P, *et al.* (2016): Optogenetic activation of adenosine A2A receptor signaling in the dorsomedial striatopallidal neurons suppresses goal-directed behavior. Neuropsychopharmacology 41:1003–1013.
67. Abel T, Zukin RS (2008): Epigenetic targets of HDAC inhibition in neurodegenerative and psychiatric disorders. Curr Opin Pharmacol 8:57–64.
68. Bagot RC, Labonté B, Peña CJ, Nestler EJ (2014): Epigenetic signaling in psychiatric disorders: Stress and depression. Dialogues Clin Neurosci 16:281–295.
69. Peña CJ, Bagot RC, Labonté B, Nestler EJ (2014): Epigenetic signaling in psychiatric disorders. J Mol Biol 426:3389–3412.
70. Robison AJ, Nestler EJ (2011): Transcriptional and epigenetic mechanisms of addiction. Nat Rev Neurosci 12:623–637.
71. Tsankova N, Renthal W, Kumar A, Nestler EJ (2007): Epigenetic regulation in psychiatric disorders. Nat Rev Neurosci 8:355–367.
72. Heindel WC, Butters N, Salmon DP (1988): Impaired learning of a motor skill in patients with Huntington's disease. Behav Neurosci 102:141–147.
73. Cohen H, Pourcher E (2007): Intact encoding, impaired consolidation in procedural learning in Parkinson's disease. Exp Brain Res 179: 703–708.
74. Agostino R, Sanes JN, Hallett M (1996): Motor skill learning in Parkinson's disease. J Neurol Sci 139:218–226.
75. Knowlton BJ, Mangels JA, Squire LR (1996): A neostriatal habit learning system in humans. Science 273:1399–1402.
76. Gillan CM, Robbins TW, Sahakian BJ, van den Heuvel OA, van Wingen G (2016): The role of habit in compulsivity. Eur Neuropsychopharmacol 26:828–840.
77. Gillan CM, Morein-Zamir S, Urcelay GP, Sule A, Voon V, Apergis-Schoute AM, *et al.* (2014): Enhanced avoidance habits in obsessive-compulsive disorder. Biol Psychiatry 75:631–638.
78. Gillan CM, Papmeyer M, Morein-Zamir S, Sahakian BJ, Fineberg NA, Robbins TW, *et al.* (2011): Disruption in the balance between goal-directed behavior and habit learning in obsessive-compulsive disorder. Am J Psychiatry 168:718–726.
79. Alvares GA, Balleine BW, Whittle L, Guastella AJ (2016): Reduced goal-directed action control in autism spectrum disorder. Autism Res 9:1285–1293.
80. Morris RW, Quail S, Griffiths KR, Green MJ, Balleine BW (2015): Corticostriatal control of goal-directed action is impaired in schizophrenia. Biol Psychiatry 77:187–195.
81. Griffiths KR, Morris RW, Balleine BW (2014): Translational studies of goal-directed action as a framework for classifying deficits across psychiatric disorders. Front Syst Neurosci 8:101.
82. Belin D, Belin-Rauscent A, Murray JE, Everitt BJ (2013): Addiction: Failure of control over maladaptive incentive habits. Curr Opin Neurobiol 23:564–572.
83. Corbit LH, Janak PH (2016): Habitual alcohol seeking: Neural bases and possible relations to alcohol use disorders. Alcohol Clin Exp Res 40:1380–1389.
84. Sebold M, Nebe S, Garbusow M, Guggenmos M, Schad DJ, Beck A, *et al.* (2017): When habits are dangerous: Alcohol expectancies and habitual decision making predict relapse in alcohol dependence. Biol Psychiatry 82:847–856.
85. Corbit LH (2016): Effects of obesogenic diets on learning and habitual responding. Curr Opin Behav Sci 9:84–90.
86. White AO, Wood MA (2014): Does stress remove the HDAC brakes for the formation and persistence of long-term memory? Neurobiol Learn Mem 112:61–67.
87. Dias-Ferreira E, Sousa JC, Melo I, Morgado P, Mesquita AR, Cerqueira JJ, *et al.* (2009): Chronic stress causes frontostriatal reorganization and affects decision-making. Science 325: 621–625.
88. Soares JM, Sampaio A, Ferreira LM, Santos NC, Marques F, Palha JA, *et al.* (2012): Stress-induced changes in human decision-making are reversible. Transl Psychiatry 2:e131.
89. Schwabe L, Wolf OT (2011): Stress-induced modulation of instrumental behavior: From goal-directed to habitual control of action. Behav Brain Res 219:321–328.
90. Malvaez M, Wassum K (2018): Regulation of habit formation in the dorsal striatum. Curr Opin Behav Sci 20:67–74.
91. Gräff J, Tsai LH (2013): The potential of HDAC inhibitors as cognitive enhancers. Annu Rev Pharmacol Toxicol 53:311–330.
92. Kelly TK, De Carvalho DD, Jones PA (2010): Epigenetic modifications as therapeutic targets. Nat Biotechnol 28:1069–1078.
93. Dietz KC, Casaccia P (2010): HDAC inhibitors and neurodegeneration: At the edge between protection and damage. Pharmacol Res 62: 11–17.
94. Hahnen E, Hauke J, Tränkle C, Eyüpoglu IY, Wirth B, Blümcke I (2008): Histone deacetylase inhibitors: Possible implications for neurodegenerative disorders. Expert Opin Investig Drugs 17:169–184.
95. Chuang DM, Leng Y, Marinova Z, Kim HJ, Chiu CT (2009): Multiple roles of HDAC inhibition in neurodegenerative conditions. Trends Neurosci 32:591–601.
96. Paxinos G, Watson C (1998): The Rat Brain in Stereotaxic Coordinates, 4th ed. San Diego, CA: Academic Press.