Habits are negatively regulated by histone deacetylase 3 in the dorsal striatum

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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 (DMS) and dorsolateral (DLS) striatum, respectively. But 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—To do this, we combined procedures that diagnose behavioral strategy in rats with pharmacological and viral-mediated HDAC manipulations, chromatin immunoprecipitation, and mRNA 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, CREB-regulated, learning-related genes in the dorsal striatum as habits formed with overtraining and with post-training HDAC inhibition. Decreasing HDAC3 function, either by selective pharmacological inhibition or expression of dominant-negative mutated HDAC3, in *either* the DLS or DMS accelerated habit formation, while HDAC3 overexpression in either region prevented habit.

Conclusions—These results challenge the strict dissociation between DMS and DLS function in goal-directed v. habitual behavioral control and identify dorsal striatal 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.

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 cortico-basal ganglia circuits centered on the dorsomedial (DMS) (7–10) and dorsolateral (DLS) (11, 12) striatum, 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. Post-translational 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 action-reward learning using procedures that diagnose behavioral strategy combined with pharmacological or viral-mediated manipulation of HDAC function and molecular analysis.

MATERIALS AND METHODS

A detailed description of the methods is provided in the Supplemental Material. Specific experiments are described below. All procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the UCLA Institutional Animal Care and Use Committee.

RESULTS

Effect of post-training 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 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-s 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) (Fig. 1A). Rats were administered the nonspecific class I HDAC inhibitor sodium butyrate (25) (NaBut, 1.0 g/kg, i.p. (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 (Fig. 1B–D; Training day: Limited, $F_{3,60}$=92.59, P<0.0001; Intermediate, $F_{4,80}$=50.69, P<0.0001; Extended, $F_{6,96}$=18.64, P<0.0001), though the NaBut group plateaued at a lower rate than vehicle controls with limited (Drug x Day: $F_{3,60}$=3.63, P=0.02), or intermediate ($F_{4,80}$=5.49, P<0.001), but not extended ($F_{6,96}$=0.42, P=0.86) training.

Behavioral strategy cannot be determined from simple lever-press performance. To identify the degree of goal-directed v. habitual control, rats were given a 5-min, drug-free, outcome-specific devaluation test (Fig. 1A). Non-reinforced lever pressing was assessed following sensory-specific satiation (1-hr pre-feeding) on the food pellet earned by lever pressing (Devalued state). This was compared to pressing after satiation on an alternate food pellet that had been non-contingently 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 v. habitual control and to control for variability in press rates between subjects (for raw press rates, see Fig. 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 (Fig. 1E; Devaluation: $F_{1,20}$=19.68, P=0.003) and a failure to reduce responding following devaluation in the extended training group (Fig. 1G; $F_{1,16}$=0.62, P>0.999). This was not altered by post-training HDAC inhibition (Drug: Limited, $F_{1,20}$=0.00, P>0.999; Extended, $F_{1,16}$=1.88, P=0.19; Drug x Devaluation: Limited, $F_{1,20}$=0.41, P=0.53; Extended, $F_{1,16}$=0.06, P=0.80), 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 may have resulted from off-target effects of extended NaBut treatment.

Post-training HDAC inhibition did, however, accelerate the rate at which habit came to dominate behavioral control. With intermediate training, NaBut-treated animals became insensitive to devaluation of the earned reward, failing to reduce responding in the Devalued

v. Valued states, while lever pressing in vehicle-treated subjects remained sensitive to devaluation (Fig. 1F; Devaluation: $F_{1,20}$=1.68, P=0.21; Drug: $F_{1,20}$=0.74, P=0.40; Drug x Devaluation: $F_{1,20}$=4.85, P=0.04). In separate groups, we found that post-training HDAC inhibition accelerated habit formation when behavioral strategy was assayed by reversal of the action-outcome contingency (Fig. S2), which does not involve a value or satiety manipulation, and occurred even when rats were trained on a random-ratio reinforcement schedule (Fig. S3) known to promote goal-directed control (1). Importantly, this effect did not manifest if NaBut was administered outside the memory-consolidation window (Fig. 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 post-training HDAC inhibition on dorsal striatal 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 (Fig. 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). 1 hr after the last intermediate training session and drug delivery, H4K8Ac was significantly higher in the DLS (t_8=3.08, P=0.02) of NaBut-treated subjects compared to controls (Fig. 1I–J, see also Fig. S5). H4K8Ac was also elevated in the DMS (t_8=2.41, P=0.04), but there were no significant differences detected in any other regions examined (see Table S1). This increase in histone acetylation was restricted to neurons in the DMS and occurred in both neuronal and non-neuronal cells in the DLS (Fig. 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 post-training HDAC inhibition (Fig. S7).

Effect of training and post-training HDAC inhibition on HDAC3 occupancy at learning-related gene promoters and gene expression in the dorsolateral striatum

By removing acetyl groups, HDACs, typically, repress gene transcription (19). That post-training HDAC inhibition accelerated the transition to habit, suggests that HDACs might 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 might 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 (ChIP) 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, involved in long-term memory formation (30, 31, 33–35), and are regulated by cAMP 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).

1 hr following the last intermediate training session (Fig. 2A), HDAC3 occupancy at the *Bdnf exon 1* promoter was enriched relative to homecage controls (*P*<0.001), but returned to control levels with extended training and was not elevated above control levels when intermediate training was followed by NaBut treatment (Fig. 2B; $F_{3,12}$=18.53, *P*<0.001). A similar pattern was detected for HDAC3 occupancy at the promoter for *Nr4a2* (Fig. 2D; $F_{3,12}$=5.74, *P*=0.01). Although there was an overall effect of training/treatment group on HDAC3 occupancy at the *Nr4a1* promoter (Fig. 2C; $F_{3,21}$=3.12, *P*=0.05), in no case was HDAC3 enrichment at this promoter significantly different from control (P>0.05). Expression of *Bdnf1* (Fig. 2E), quantified with qPCR, was significantly elevated 1 hr following intermediate training with NaBut treatment relative to homecage controls (t_{13}=2.41, *P*=0.03), the intermediate trained control group (t_{13}=3.02, *P*=0.01), and controls following extended training (t_{13}=3.23, *P*=0.01). A similar pattern was detected in *Bdnf9* expression (Fig. S8). *Nr4a1* and *Nr4a2* expression (Fig. 2F–G) were not significantly different from controls 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. Post-training 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 dorsolateral striatum 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 might promote habit formation. We tested this hypothesis in two ways. First, by pharmacologically inhibiting HDAC3 activity specifically in the DLS immediately after each intermediate instrumental training session and, second, by expressing a dominant negative HDAC3 point mutant (34) in the DLS prior to all training to selectively disrupt HDAC3 enzymatic activity, without affecting protein-protein interactions (40, 41) (Fig. 3A). In both cases, habit formation was potentiated, recapitulating the systemic HDAC inhibition result. Post-training intra-DLS infusion of the selective HDAC3 inhibitor RGFP966 (1.0 ng/0.5μl/side) (31, 42, 43) elevated H4K8Ac in the DLS (Fig. 3B–C; t_8=3.15, *P*=0.014; see also Fig. S9), but not the adjacent DMS (Fig. 3B; Normalized H4K8Ac optical density: Vehicle, 1.011±0.10; RGFP966, 1.00±0.27; t_3=0.06, *P*=0.954) compared to vehicle-treated controls. This treatment did not alter the acquisition of instrumental lever-press behavior (Fig. 3D; Training

day: $F_{4,68}=32.61$, $P<0.001$; Drug: $F_{1,17}=0.95$, $P=0.34$; Drug x Day: $F_{4,68}=2.32$, $P=0.07$), but did render this behavior insensitive to devaluation of the earned reward under conditions in which intermediate-trained controls remained sensitive (Fig. 3E; Devaluation: $F_{1,17}=10.83$, $P=0.004$; Drug: $F_{1,17}=1.12$, $P=0.31$; Drug x Devaluation: $F_{1,17}=7.25$, $P=0.02$). Similarly, expressing a dominant negative point mutant of HDAC3 (AAV2/1-CMV-HDAC3Y298H-V5), in the DLS produced H4K8 hyperacetylation (Fig. 3F–G; $t_{10}=3.91$, $P=0.003$) and insensitivity to outcome devaluation (Fig. 3I; Devaluation: $F_{1,14}=3.74$ $P=0.07$; Virus: $F_{1,14}=0.47$, $P=0.506$; Virus x Devaluation: $F_{1,17}=6.19$, $P=0.03$), without altering lever-pressing acquisition (Fig. 3H; Training day: $F_{4,56}=29.21$, $P<0.001$; Virus: $F_{1,14}<0.01$, $P=0.99$; Virus x Day: $F_{4,56}=0.56$, $P=0.69$). These results demonstrate that attenuating DLS HDAC3 activity promotes habit formation.

The combined data strongly suggest that in the DLS HDAC3 might 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 (Fig. 3J–K; $t_9=2.29$, $P=0.048$), and gave subjects extended instrumental training known to promote habit. All subjects similarly acquired the lever-press behavior (Fig. 3L; Training day: $F_{6,126}=81.44$, $P<0.001$; Virus: $F_{1,21}=0.06$, $P=0.81$; Virus x Day: $F_{6,126}=0.44$, $P=0.85$). 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 (Fig. 3M; Devaluation: $F_{1,21}=2.24$ $P=0.15$; Virus: $F_{1,21}=-8.17$, $P>0.999$; Virus x Devaluation: $F_{1,21}=6.45$, $P=0.02$), even after extensive overtraining (Fig. S10). Together, these data demonstrate that in the DLS HDAC3 is a critical negative regulator of habit formation.

Effect of HDAC3 manipulation in dorsomedial striatum 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 (Fig. 4A). Both post-training intra-DMS HDAC3 inhibition (RGFP966, 1.0 ng/0.5μl/side; Fig. 4B–C; DMS: $t_7=2.54$, $P=0.04$; DLS: Vehicle, 1.00±0.02 normalized H4K8Ac optical density v. RGFP966 1.09±0.07, $t_7=1.30$, $P=0.23$; see also Fig. S11) and expression of the dominant negative HDAC3 point mutant in the DMS (Fig. 4F–G; $t_7=4.32$, $P=0.004$) induced a hyperacetylated H4K8 state that was restricted to the DMS. Neither treatment affected acquisition of the lever-press behavior (RGFP966: Fig. 4D; Training day: $F_{4,64}=45.38$, $P<0.001$; Drug: $F_{1,16}=0.06$, $P=0.81$; Drug x Day; $F_{4,64}=0.12$, $P=0.98$; HDAC3pm: Fig. 4H; Day: $F_{4,84}=32.38$, $P<0.001$; Virus: $F_{1,21}=0.19$ $P=0.67$; Virus x Day: $F_{4,84}=0.44$, $P=0.78$). To our surprise, given the canonical function of the DMS in goal-directed, not habit, learning (7, 8), both post-training intra-DMS RGFP966 (Fig. 4E; Devaluation: $F_{1,16}=1.16$ $P=0.298$; Drug: $F_{1,16}=0.00$, $P>0.999$; Drug x Devaluation: $F_{1,16}=4.79$, $P=0.04$) and DMS HDAC3 point mutant expression (Fig. 4I; Devaluation: $F_{1,21}=1.94$, $P=0.18$; Virus: $F_{1,21}=17.61$, $P<0.001$; Virus x Devaluation: $F_{1,21}=5.52$, $P=0.03$)

potentiated habit formation, as indicated by insensitivity to devaluation of the earned reward following intermediate training. Conversely, DMS HDAC3 overexpression reduced H4K8Ac (Fig. 4J–K; t_8=3.27, P=0.01) did not alter lever pressing acquisition (Fig. 3L; Day: $F_{6,132}$=61.69, P<0.001; Virus: $F_{1,22}$=1.36, P=0.26; Virus x Day: $F_{6,132}$=2.01, P=0.07), but prevented habit formation (Fig. 4M; Devaluation: $F_{1,22}$=7.06, P=0.01; Virus: $F_{1,22}$=2.68, P=0.12; Virus x Devaluation: $F_{1,22}$=4.34, P=0.049), even with extensive overtraining (Fig. S12). These unexpected results demonstrate that, like 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 ChIP coupled with qPCR (Fig 5A), we found that, unlike in the DLS, HDAC3 occupancy at *Bdnf1* was not significantly altered 1 hr following instrumental training or intermediate training followed by systemic NaBut treatment (Fig. 5B; $F_{3,22}$=1.87, P=0.16). Similarly, in no case was HDAC3 enrichment at the *Nr4a2* promoter significantly different from controls (Fig. 5D; $F_{3,21}$=2.74, P=0.07). Instead, HDAC3 occupancy at the *Nr4a1* promoter (Fig. 5C; $F_{3,12}$=7.76, P=0.004) was increased relative to homecage controls with intermediate training (P<0.05) and returned to control levels following extended training and was not elevated following intermediate training with systemic NaBut treatment. Follow up mRNA analyses (Fig. 5E–G) revealed *Nr4a2* expression (Fig. 5G; $F_{3,31}$=3.45, P=0.03) was attenuated relative to homecage controls with intermediate training (P<0.01) and intermediate training with systemic NaBut treatment (P<0.05). *Bdnf1* and *Nr4a1* were not significantly altered (Fig. 5E; *Bdnf1*, $F_{3,16}$=1.57, P=0.24; see also Fig. S8; *Nr4a1*, $F_{3,25}$=1.87, P=0.55). 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 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 dorsal striatal 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 post-training 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 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 form 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 dorsal 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 post-training 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 co-repressor 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, like HDAC3, and activating enzymes, such as transcription factors and HATs. Recruitment of HATs, such as CREB-binding protein (28, 36), are required for HDAC3 regulation of gene transcription and memory. Moreover, it is also possible that the non-specific class I HDAC inhibition treatment engaged repressive mechanisms (53) additional to those normally engaged to regulate gene expression differently than learning alone, perhaps suggesting alternate, non-natural 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 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, provide 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 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 both the anterior and posterior DMS to be 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 in which has been shown to promote habit (66). In either case, the distinct HDAC3 occupancy patterns at learning-related gene promoters in the DLS v. DMS suggests 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. Dorsal 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 (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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

References

1. Dickinson A. Actions and Habits: the development of behavioural autonomy. Philosphical Transactions of the Royal Society of London. 1985; B308:67–78.

2. Dolan RJ, Dayan P. Goals and habits in the brain. Neuron. 2013; 80:312–325. [PubMed: 24139036]

3. Adams CD, Dickinson A. Instrumental responding following reinforcer devaluation. The Quarterly Journal of Experimental Psychology. 1981; 33:109–121.

4. Adams CD. Variations in the sensitivity of instrumental responding to reinforcer devaluation. Quarterly Journal of Experimental Psychology. 1982; 34:77–98.

5. Gillan CM, Kosinski M, Whelan R, Phelps EA, Daw ND. Characterizing a psychiatric symptom dimension related to deficits in goal-directed control. Elife. 2016:5.

6. Voon V, Derbyshire K, Rück C, Irvine MA, Worbe Y, Enander J, et al. Disorders of compulsivity: a common bias towards learning habits. Mol Psychiatry. 2015; 20:345–352. [PubMed: 24840709]

7. Yin HH, Knowlton BJ, Balleine BW. Blockade of NMDA receptors in the dorsomedial striatum prevents action-outcome learning in instrumental conditioning. Eur J Neurosci. 2005; 22:505–512. [PubMed: 16045503]

8. Yin HH, Ostlund SB, Knowlton BJ, Balleine BW. The role of the dorsomedial striatum in instrumental conditioning. Eur J Neurosci. 2005; 22:513–523. [PubMed: 16045504]

9. Corbit LH, Janak PH. Posterior dorsomedial striatum is critical for both selective instrumental and Pavlovian reward learning. Eur J Neurosci. 2010; 31:1312–1321. [PubMed: 20345912]

10. Liljeholm M, Tricomi E, O'Doherty JP, Balleine BW. Neural correlates of instrumental contingency learning: differential effects of action-reward conjunction and disjunction. J Neurosci. 2011; 31:2474–2480. [PubMed: 21325514]

11. Yin HH, Knowlton BJ, Balleine BW. Lesions of dorsolateral striatum preserve outcome expectancy but disrupt habit formation in instrumental learning. Eur J Neurosci. 2004; 19:181–189. [PubMed: 14750976]

12. Tricomi E, Balleine BW, O'Doherty JP. A specific role for posterior dorsolateral striatum in human habit learning. Eur J Neurosci. 2009; 29:2225–2232. [PubMed: 19490086]

13. Pittenger C, Fasano S, Mazzocchi-Jones D, Dunnett SB, Kandel ER, Brambilla R. Impaired bidirectional synaptic plasticity and procedural memory formation in striatum-specific cAMP response element-binding protein-deficient mice. J Neurosci. 2006; 26:2808–2813. [PubMed: 16525060]

14. Hernandez A, Tan C, Mettlach G, Pozo K, Plattner F, Bibb JA. Cdk5 Modulates Long-Term Synaptic Plasticity and Motor Learning in Dorsolateral Striatum. Scientific Reports. 2016; 6:29812. [PubMed: 27443506]

15. Shiflett MW, Brown RA, Balleine BW. Acquisition and performance of goal-directed instrumental actions depends on ERK signaling in distinct regions of dorsal striatum in rats. J Neurosci. 2010; 30:2951–2959. [PubMed: 20181592]

16. Brightwell JJ, Smith CA, Neve RL, Colombo PJ. Transfection of mutant CREB in the striatum, but not the hippocampus, impairs long-term memory for response learning. Neurobiol Learn Mem. 2008; 89:27–35. [PubMed: 17977028]

17. Colombo PJ, Brightwell JJ, Countryman RA. Cognitive strategy-specific increases in phosphorylated cAMP response element-binding protein and c-Fos in the hippocampus and dorsal striatum. J Neurosci. 2003; 23:3547–3554. [PubMed: 12716964]

18. Hernandez PJ, Schiltz CA, Kelley AE. 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. 2006; 13:599–608. [PubMed: 17015857]

19. Kouzarides T. Chromatin modifications and their function. Cell. 2007; 128:693–705. [PubMed: 17320507]

20. Day JJ, Sweatt JD. Epigenetic mechanisms in cognition. Neuron. 2011; 70:813–829. [PubMed: 21658577]

21. Peixoto L, Abel T. The role of histone acetylation in memory formation and cognitive impairments. Neuropsychopharmacology. 2013; 38:62–76. [PubMed: 22669172]

22. Vogel-Ciernia A, Wood MA. Molecular brake pad hypothesis: pulling off the brakes for emotional memory. Rev Neurosci. 2012; 23:607–626. [PubMed: 23096102]

23. McQuown SC, Wood MA. HDAC3 and the molecular brake pad hypothesis. Neurobiol Learn Mem. 2011; 96:27–34. [PubMed: 21521655]

24. Borrelli E, Nestler EJ, Allis CD, Sassone-Corsi P. Decoding the epigenetic language of neuronal plasticity. Neuron. 2008; 60:961–974. [PubMed: 19109904]

25. Kilgore M, Miller CA, Fass DM, Hennig KM, Haggarty SJ, Sweatt JD, et al. Inhibitors of class 1 histone deacetylases reverse contextual memory deficits in a mouse model of Alzheimer's disease. Neuropsychopharmacology. 2010; 35:870–880. [PubMed: 20010553]

26. Malvaez M, Sanchis-Segura C, Vo D, Lattal KM, Wood MA. Modulation of chromatin modification facilitates extinction of cocaine-induced conditioned place preference. Biol Psychiatry. 2010; 67:36–43. [PubMed: 19765687]

27. Lattal KM, Barrett RM, Wood MA. Systemic or intrahippocampal delivery of histone deacetylase inhibitors facilitates fear extinction. Behav Neurosci. 2007; 121:1125–1131. [PubMed: 17907845]

28. Stefanko DP, Barrett RM, Ly AR, Reolon GK, Wood MA. Modulation of long-term memory for object recognition via HDAC inhibition. Proc Natl Acad Sci U S A. 2009; 106:9447–9452. [PubMed: 19470462]

29. Ploense KL, Kerstetter KA, Wade MA, Woodward NC, Maliniak D, Reyes M, et al. Exposure to histone deacetylase inhibitors during Pavlovian conditioning enhances subsequent cue-induced reinstatement of operant behavior. Behav Pharmacol. 2013; 24:164–171. [PubMed: 23604166]

30. Bredy TW, Wu H, Crego C, Zellhoefer J, Sun YE, Barad M. Histone modifications around individual BDNF gene promoters in prefrontal cortex are associated with extinction of conditioned fear. Learn Mem. 2007; 14:268–276. [PubMed: 17522015]

31. Malvaez M, McQuown SC, Rogge GA, Astarabadi M, Jacques V, Carreiro S, et al. HDAC3-selective inhibitor enhances extinction of cocaine-seeking behavior in a persistent manner. Proc Natl Acad Sci U S A. 2013; 110:2647–2652. [PubMed: 23297220]

32. Malvaez M, Mhillaj E, Matheos DP, Palmery M, Wood MA. CBP in the nucleus accumbens regulates cocaine-induced histone acetylation and is critical for cocaine-associated behaviors. J Neurosci. 2011; 31:16941–16948. [PubMed: 22114264]

33. McQuown SC, Barrett RM, Matheos DP, Post RJ, Rogge GA, Alenghat T, et al. HDAC3 is a critical negative regulator of long-term memory formation. J Neurosci. 2011; 31:764–774. [PubMed: 21228185]

34. Kwapis JL, Alaghband Y, López AJ, White AO, Campbell RR, Dang RT, et al. Context and Auditory Fear are Differentially Regulated by HDAC3 Activity in the Lateral and Basal Subnuclei of the Amygdala. Neuropsychopharmacology. 2017; 42:1284–1294. [PubMed: 27924874]

35. Intlekofer KA, Berchtold NC, Malvaez M, Carlos AJ, McQuown SC, Cunningham MJ, et al. Exercise and sodium butyrate transform a subthreshold learning event into long-term memory via a brain-derived neurotrophic factor-dependent mechanism. Neuropsychopharmacology. 2013; 38:2027–2034. [PubMed: 23615664]

36. Vecsey CG, Hawk JD, Lattal KM, Stein JM, Fabian SA, Attner MA, et al. Histone deacetylase inhibitors enhance memory and synaptic plasticity via CREB:CBP-dependent transcriptional activation. J Neurosci. 2007; 27:6128–6140. [PubMed: 17553985]

37. McNulty SE, Barrett RM, Vogel-Ciernia A, Malvaez M, Hernandez N, Davatolhagh MF, et al. Differential roles for Nr4a1 and Nr4a2 in object location vs. object recognition long-term memory. Learn Mem. 2012; 19:588–592. [PubMed: 23161447]

38. Ou LC, Gean PW. Transcriptional regulation of brain-derived neurotrophic factor in the amygdala during consolidation of fear memory. Mol Pharmacol. 2007; 72:350–358. [PubMed: 17456785]

39. Broide RS, Redwine JM, Aftahi N, Young W, Bloom FE, Winrow CJ. Distribution of histone deacetylases 1–11 in the rat brain. J Mol Neurosci. 2007; 31:47–58. [PubMed: 17416969]

40. Lahm A, Paolini C, Pallaoro M, Nardi MC, Jones P, Neddermann P, et al. Unraveling the hidden catalytic activity of vertebrate class IIa histone deacetylases. Proc Natl Acad Sci U S A. 2007; 104:17335–17340. [PubMed: 17956988]

41. Sun Z, Feng D, Fang B, Mullican SE, You SH, Lim HW, et al. Deacetylase-independent function of HDAC3 in transcription and metabolism requires nuclear receptor corepressor. Mol Cell. 2013; 52:769–782. [PubMed: 24268577]

42. Rumbaugh G, Sillivan SE, Ozkan ED, Rojas CS, Hubbs CR, Aceti M, et al. Pharmacological Selectivity Within Class I Histone Deacetylases Predicts Effects on Synaptic Function and Memory Rescue. Neuropsychopharmacology. 2015; 40:2307–2316. [PubMed: 25837283]

43. Bieszczad KM, Bechay K, Rusche JR, Jacques V, Kudugunti S, Miao W, et al. Histone Deacetylase Inhibition via RGFP966 Releases the Brakes on Sensory Cortical Plasticity and the Specificity of Memory Formation. J Neurosci. 2015; 35:13124–13132. [PubMed: 26400942]

44. Alberini CM. Transcription factors in long-term memory and synaptic plasticity. Physiol Rev. 2009; 89:121–145. [PubMed: 19126756]

45. Bourtchuladze R, Frenguelli B, Blendy J, Cioffi D, Schutz G, Silva AJ. Deficient long-term memory in mice with a targeted mutation of the cAMP-responsive element-binding protein. Cell. 1994; 79:59–68. [PubMed: 7923378]

46. Yin JC, Wallach JS, Del Vecchio M, Wilder EL, Zhou H, Quinn WG, et al. Induction of a dominant negative CREB transgene specifically blocks long-term memory in Drosophila. Cell. 1994; 79:49–58. [PubMed: 7923376]

47. Guzowski JF, McGaugh JL. 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. 1997; 94:2693–2698. [PubMed: 9122258]

48. Pittenger C, Huang YY, Paletzki RF, Bourtchouladze R, Scanlin H, Vronskaya S, et al. Reversible inhibition of CREB/ATF transcription factors in region CA1 of the dorsal hippocampus disrupts hippocampus-dependent spatial memory. Neuron. 2002; 34:447–462. [PubMed: 11988175]

49. Tao X, Finkbeiner S, Arnold DB, Shaywitz AJ, Greenberg ME. Ca2+ influx regulates BDNF transcription by a CREB family transcription factor-dependent mechanism. Neuron. 1998; 20:709–726. [PubMed: 9581763]

50. Lu Y, Christian K, Lu B. BDNF: a key regulator for protein synthesis-dependent LTP and long-term memory? Neurobiol Learn Mem. 2008; 89:312–323. [PubMed: 17942328]

51. White AO, Kramár EA, López AJ, Kwapis JL, Doan J, Saldana D, et al. BDNF rescues BAF53b-dependent synaptic plasticity and cocaine-associated memory in the nucleus accumbens. Nat Commun. 2016; 7:11725. [PubMed: 27226355]

52. Gottmann K, Mittmann T, Lessmann V. BDNF signaling in the formation, maturation and plasticity of glutamatergic and GABAergic synapses. Exp Brain Res. 2009; 199:203–234. [PubMed: 19777221]

53. Kennedy PJ, Feng J, Robison AJ, Maze I, Badimon A, Mouzon E, et al. Class I HDAC inhibition blocks cocaine-induced plasticity by targeted changes in histone methylation. Nat Neurosci. 2013; 16:434–440. [PubMed: 23475113]

54. Balleine BW, Delgado MR, Hikosaka O. The role of the dorsal striatum in reward and decision-making. J Neurosci. 2007; 27:8161–8165. [PubMed: 17670959]

55. Balleine BW, O'Doherty JP. Human and rodent homologies in action control: corticostriatal determinants of goal-directed and habitual action. Neuropsychopharmacology. 2010; 35:48–69. [PubMed: 19776734]

56. O'Hare JK, Ade KK, Sukharnikova T, Van Hooser SD, Palmeri ML, Yin HH, et al. Pathway-Specific Striatal Substrates for Habitual Behavior. Neuron. 2016; 89:472–479. [PubMed: 26804995]

57. Shan Q, Christie MJ, Balleine BW. Plasticity in striatopallidal projection neurons mediates the acquisition of habitual actions. Eur J Neurosci. 2015; 42:2097–2104. [PubMed: 26054036]

58. Smith KS, Graybiel AM. A dual operator view of habitual behavior reflecting cortical and striatal dynamics. Neuron. 2013; 79:361–374. [PubMed: 23810540]

59. Rueda-Orozco PE, Robbe D. The striatum multiplexes contextual and kinematic information to constrain motor habits execution. Nat Neurosci. 2015; 18:453–460. [PubMed: 25622144]

60. Shan Q, Ge M, Christie MJ, Balleine BW. The acquisition of goal-directed actions generates opposing plasticity in direct and indirect pathways in dorsomedial striatum. J Neurosci. 2014; 34:9196–9201. [PubMed: 25009253]

61. Gremel CM, Costa RM. Orbitofrontal and striatal circuits dynamically encode the shift between goal-directed and habitual actions. Nat Commun. 2013; 4:2264. [PubMed: 23921250]

62. Gremel CM, Chancey JH, Atwood BK, Luo G, Neve R, Ramakrishnan C, et al. Endocannabinoid Modulation of Orbitostriatal Circuits Gates Habit Formation. Neuron. 2016; 90:1312–1324. [PubMed: 27238866]

63. Smith KS, Graybiel AM. Habit formation. Dialogues Clin Neurosci. 2016; 18:33–43. [PubMed: 27069378]

64. Graybiel AM. The basal ganglia and chunking of action repertoires. Neurobiol Learn Mem. 1998; 70:119–136. [PubMed: 9753592]

65. Thorn CA, Atallah H, Howe M, Graybiel AM. Differential dynamics of activity changes in dorsolateral and dorsomedial striatal loops during learning. Neuron. 2010; 66:781–795. [PubMed: 20547134]

66. Li Y, He Y, Chen M, Pu Z, Chen L, Li P, et al. Optogenetic Activation of Adenosine A2A Receptor Signaling in the Dorsomedial Striatopallidal Neurons Suppresses Goal-Directed Behavior. Neuropsychopharmacology. 2016; 41:1003–1013. [PubMed: 26216520]

67. Abel T, Zukin RS. Epigenetic targets of HDAC inhibition in neurodegenerative and psychiatric disorders. Curr Opin Pharmacol. 2008; 8:57–64. [PubMed: 18206423]

68. Bagot RC, Labonté B, Peña CJ, Nestler EJ. Epigenetic signaling in psychiatric disorders: stress and depression. Dialogues Clin Neurosci. 2014; 16:281–295. [PubMed: 25364280]

69. Peña CJ, Bagot RC, Labonté B, Nestler EJ. Epigenetic signaling in psychiatric disorders. J Mol Biol. 2014; 426:3389–3412. [PubMed: 24709417]

70. Robison AJ, Nestler EJ. Transcriptional and epigenetic mechanisms of addiction. Nat Rev Neurosci. 2011; 12:623–637. [PubMed: 21989194]

71. Tsankova N, Renthal W, Kumar A, Nestler EJ. Epigenetic regulation in psychiatric disorders. Nat Rev Neurosci. 2007; 8:355–367. [PubMed: 17453016]

72. Heindel WC, Butters N, Salmon DP. Impaired learning of a motor skill in patients with Huntington's disease. Behav Neurosci. 1988; 102:141–147. [PubMed: 2965592]

73. Cohen H, Pourcher E. Intact encoding, impaired consolidation in procedural learning in Parkinson's disease. Exp Brain Res. 2007; 179:703–708. [PubMed: 17279386]

74. Agostino R, Sanes JN, Hallett M. Motor skill learning in Parkinson's disease. J Neurol Sci. 1996; 139:218–226. [PubMed: 8856656]

75. Knowlton BJ, Mangels JA, Squire LR. A neostriatal habit learning system in humans. Science. 1996; 273:1399–1402. [PubMed: 8703077]

76. Gillan CM, Robbins TW, Sahakian BJ, van den Heuvel OA, van Wingen G. The role of habit in compulsivity. Eur Neuropsychopharmacol. 2016; 26:828–840. [PubMed: 26774661]

77. Gillan CM, Morein-Zamir S, Urcelay GP, Sule A, Voon V, Apergis-Schoute AM, et al. Enhanced avoidance habits in obsessive-compulsive disorder. Biol Psychiatry. 2014; 75:631–638. [PubMed: 23510580]

78. Gillan CM, Papmeyer M, Morein-Zamir S, Sahakian BJ, Fineberg NA, Robbins TW, et al. Disruption in the balance between goal-directed behavior and habit learning in obsessive-compulsive disorder. Am J Psychiatry. 2011; 168:718–726. [PubMed: 21572165]

79. Alvares GA, Balleine BW, Whittle L, Guastella AJ. Reduced goal-directed action control in autism spectrum disorder. Autism Res. 2016

80. Morris RW, Quail S, Griffiths KR, Green MJ, Balleine BW. Corticostriatal control of goal-directed action is impaired in schizophrenia. Biol Psychiatry. 2015; 77:187–195. [PubMed: 25062683]

81. Griffiths KR, Morris RW, Balleine BW. Translational studies of goal-directed action as a framework for classifying deficits across psychiatric disorders. Front Syst Neurosci. 2014; 8:101. [PubMed: 24904322]

82. Belin D, Belin-Rauscent A, Murray JE, Everitt BJ. Addiction: failure of control over maladaptive incentive habits. Curr Opin Neurobiol. 2013

83. Corbit LH, Janak PH. Habitual Alcohol Seeking: Neural Bases and Possible Relations to Alcohol Use Disorders. Alcohol Clin Exp Res. 2016

84. Sebold M, Nebe S, Garbusow M, Guggenmos M, Schad DJ, Beck A, et al. When Habits Are Dangerous: Alcohol Expectancies and Habitual Decision Making Predict Relapse in Alcohol Dependence. Biol Psychiatry. 2017; 82:847–856. [PubMed: 28673442]

85. Corbit LH. Effects of obesogenic diets on learning and habitual responding. Current Opinion in Behavioral Sciences. 2016; 9:84–90.

86. White AO, Wood MA. Does stress remove the HDAC brakes for the formation and persistence of long-term memory? Neurobiol Learn Mem. 2014; 112:61–67. [PubMed: 24149059]

87. Dias-Ferreira E, Sousa JC, Melo I, Morgado P, Mesquita AR, Cerqueira JJ, et al. Chronic stress causes frontostriatal reorganization and affects decision-making. Science. 2009; 325:621–625. [PubMed: 19644122]

88. Soares JM, Sampaio A, Ferreira LM, Santos NC, Marques F, Palha JA, et al. Stress-induced changes in human decision-making are reversible. Transl Psychiatry. 2012; 2:e131. [PubMed: 22760555]

89. Schwabe L, Wolf OT. Stress-induced modulation of instrumental behavior: from goal-directed to habitual control of action. Behav Brain Res. 2011; 219:321–328. [PubMed: 21219935]

90. Malvaez M, Wassum K. Regulation of habit formation in the dorsal striatum. Current Opinion in Behavioral Sciences. 2018; 20:67–74. [PubMed: 29713658]

91. Gräff J, Tsai LH. The potential of HDAC inhibitors as cognitive enhancers. Annu Rev Pharmacol Toxicol. 2013; 53:311–330. [PubMed: 23294310]

92. Kelly TK, De Carvalho DD, Jones PA. Epigenetic modifications as therapeutic targets. Nat Biotechnol. 2010; 28:1069–1078. [PubMed: 20944599]

93. Dietz KC, Casaccia P. HDAC inhibitors and neurodegeneration: at the edge between protection and damage. Pharmacol Res. 2010; 62:11–17. [PubMed: 20123018]

94. Hahnen E, Hauke J, Tränkle C, Eyüpoglu IY, Wirth B, Blümcke I. Histone deacetylase inhibitors: possible implications for neurodegenerative disorders. Expert Opin Investig Drugs. 2008; 17:169–184.

95. Chuang DM, Leng Y, Marinova Z, Kim HJ, Chiu CT. Multiple roles of HDAC inhibition in neurodegenerative conditions. Trends Neurosci. 2009; 32:591–601. [PubMed: 19775759]

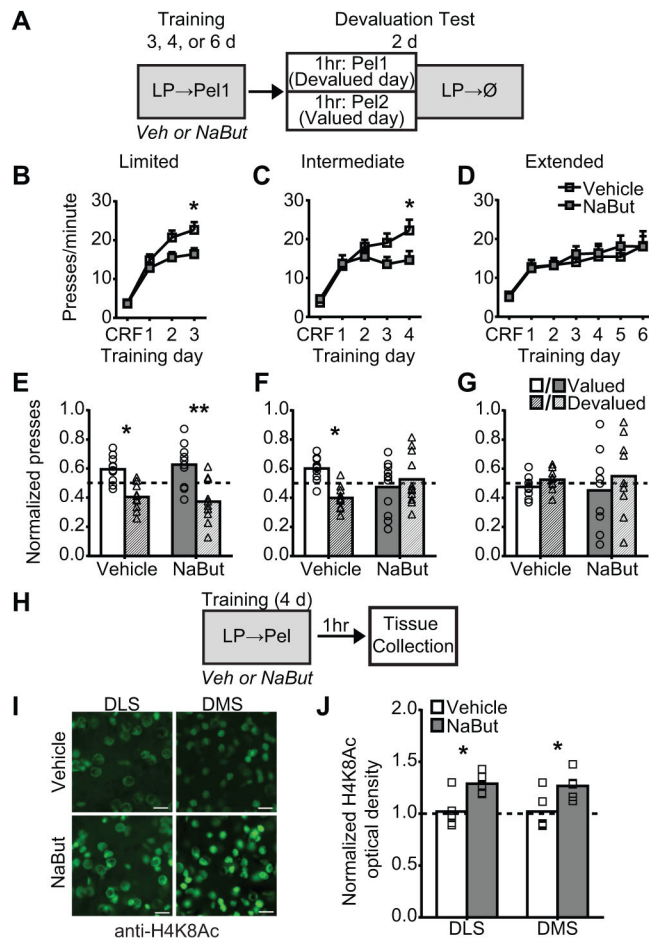96. Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 4. Academic Press; 1998.

Figure 1. Effect of post-training HDAC 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 limited (**B**; N=11/group), intermediate (**C**; N=11–12/group), or extended (**D**; N=9/group) instrumental training. (data presented as mean + s.e.m). CRF (continuous reinforcement) on the first training day lever pressing was continuously reinforced with food-pellet delivery. A RI-30s 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 limited (**E**), intermediate (**F**), or extended (**G**) training. Dashed line indicates point of equal responding between tests. (data presented as mean + scatter). (**H**) Schematic representation of procedures, (**I**) Representative immunofluorescent image and quantification (**J**) of acetylation of H4K8 (H4K8Ac; N=4–6/condition) 1 hr following instrumental training/drug treatment. Scale bar = 20 μm. Data normalized to vehicle control (dashed line). *P<0.05; ** P<0.01.
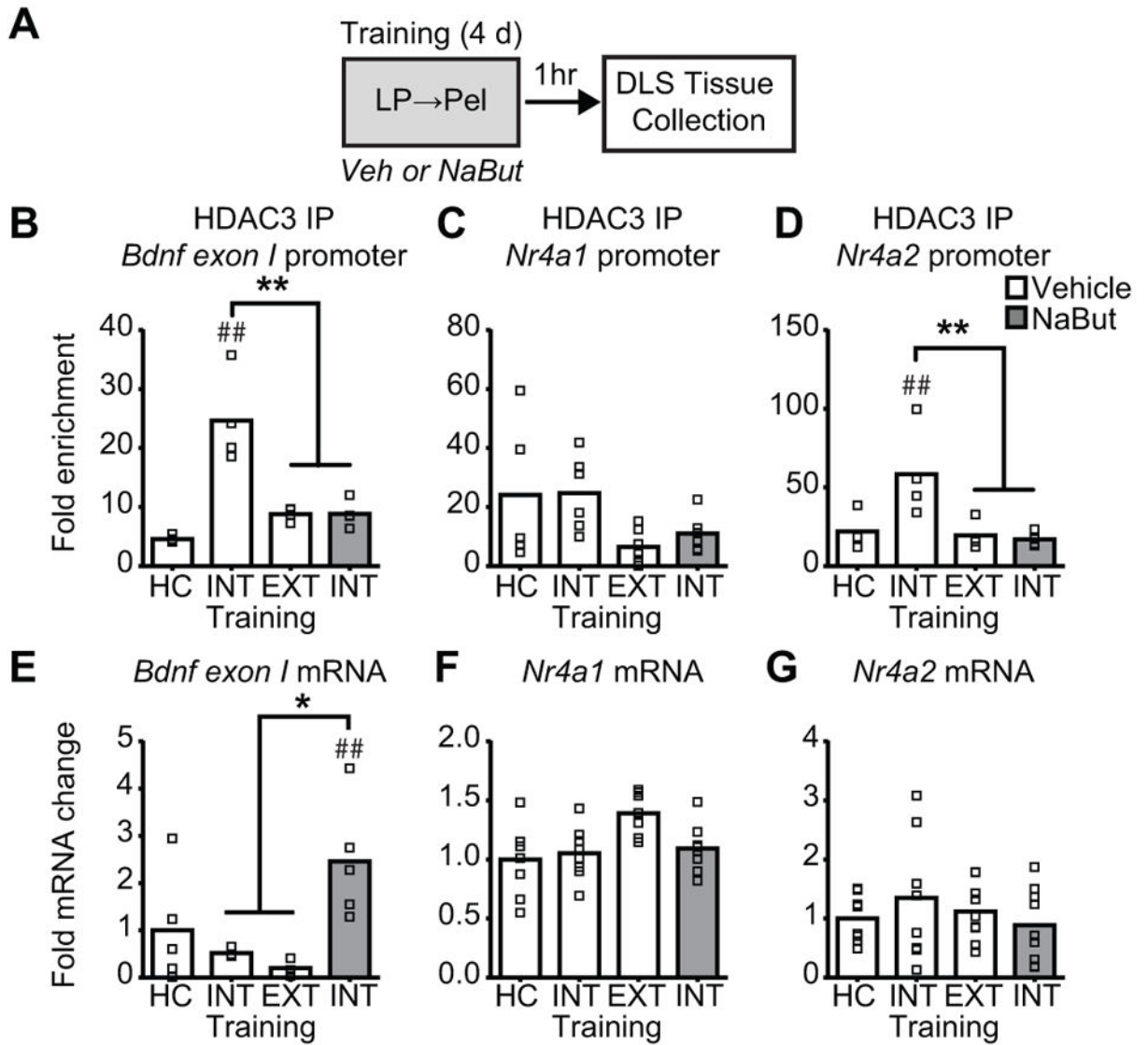
Figure 2. Effect of training and post-training HDAC inhibition on HDAC3 occupancy at learning-related gene promoters and gene expression in the dorsolateral striatum
(**A**) Schematic representation of procedures. (**B–D**) ChIP was performed with anti-HDAC3 followed by qPCR to identify HDAC3 binding to the *Bdnf1* (**B**), *Nr4a1* (**C**), *or Nr4a2* (**D**) promoters in the DLS of home cage (HC) controls or following either intermediate (INT) or extended training (EXT) in vehicle-treated rats, or NaBut treatment post-intermediate training. Data presented as fold change relative to IgG (% Input/IgG). (**E–G**) mRNA expression of *Bdnf1* (**E**), *Nr4a1* (**F**), and *Nr4a2* (**G**) in the DLS. *P<0.05, **P<0.01, between groups; ##P<0.01 relative to HC.
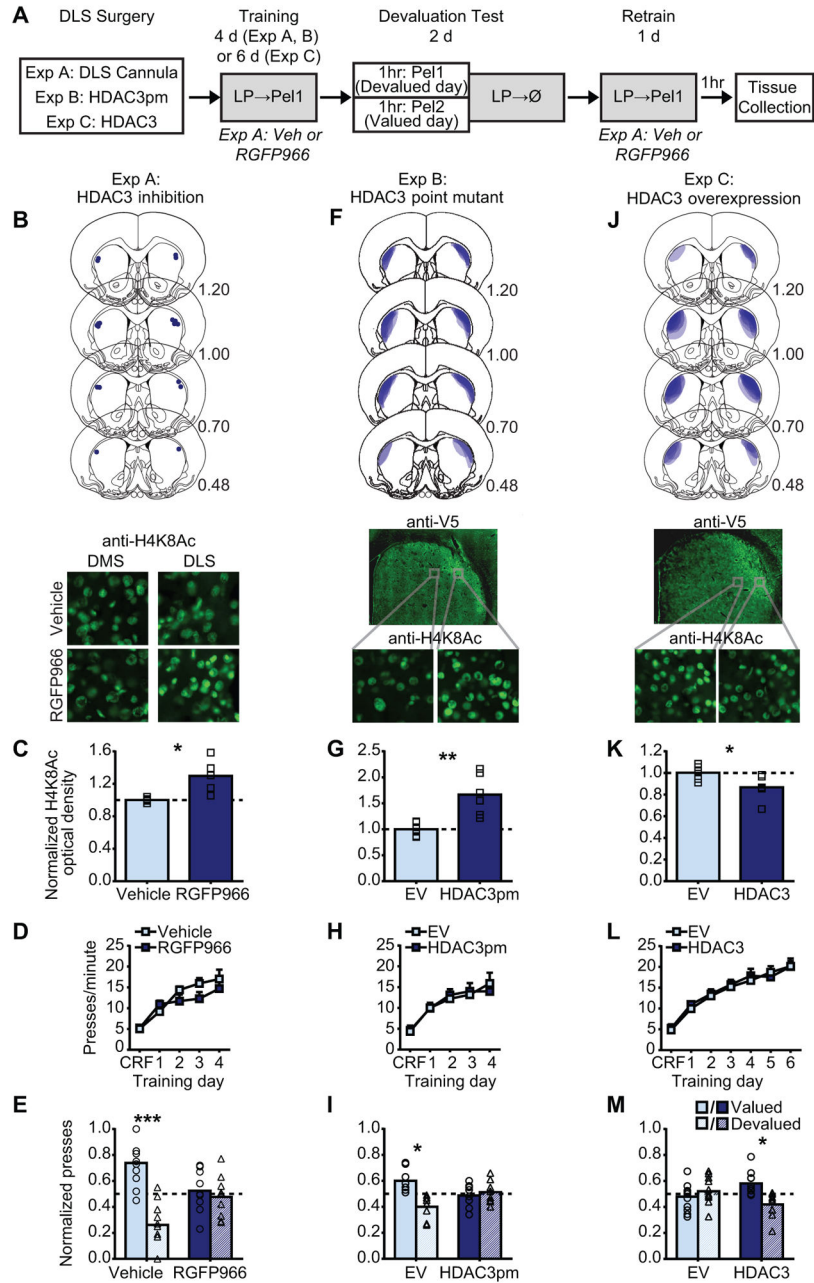
Figure 3. Effect of HDAC3 manipulation in dorsolateral striatum on habit formation

(**A**) Schematic representation of procedures. (**B**) *Top*, 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 (96). *Middle*, representative immunofluorescent images of H4K8Ac in DMS (*left*) and DLS (*right*) 1 hr following instrumental training/intra-DLS vehicle (*top*) or RGFP966 (*bottom*) infusion. Scale bar = 20 μm. (**F, J**) *Top*, schematic representation of HDAC3 point mutant (**F**), or HDAC3 (**J**) expression in the DLS for all subjects. *Middle*, representative immunofluorescent images of V5-tagged HDAC3 point mutant (**F**) or HDAC3 (**J**) expression in the DLS. *Bottom*,

Representative immunofluorescent images of H4K8Ac in rats expressing the HDAC3 point mutant (**F**) or HDAC3 (**J**) either outside (*left*) or inside (*right*) of expression zone. (**C, G, K**) Quantification of H4K8Ac for rats receiving intra-DLS vehicle or RGFP966 infusion (**C**; *N*=5/group), intra-DLS empty vector (EV) or HDAC3 point mutant (HDAC3pm) (**G**; *N*=6/ group), or intra-DLS empty vector or HDAC3 overexpression (**K**; *N*=5–6/group). (data presented as mean + scatter). (**D, H, L**) Instrumental training performance for rats given post-training intra-DLS vehicle or RGFP966 infusions (**D**; *N*=10–12/group), intra-DLS empty vector or HDAC3 point mutant (**H**; *N*=8/group), or intra-DLS empty vector or HDAC3 (**L**; *N*=11–13/group). (data presented as mean + s.e.m). CRF (continuous reinforcement) on the first training day lever pressing was continuously reinforced with food-pellet delivery. (**E, I, M**) Normalized lever presses during the subsequent devaluation tests for rats given post-training intra-DLS vehicle or RGFP966 infusions (**E**), intra-DLS empty vector (EV) or HDAC3 point mutant (**I**), or intra-DLS empty vector (EV)or HDAC3 (**M**). *P<0.05; **P<0.01; ***P<0.001.
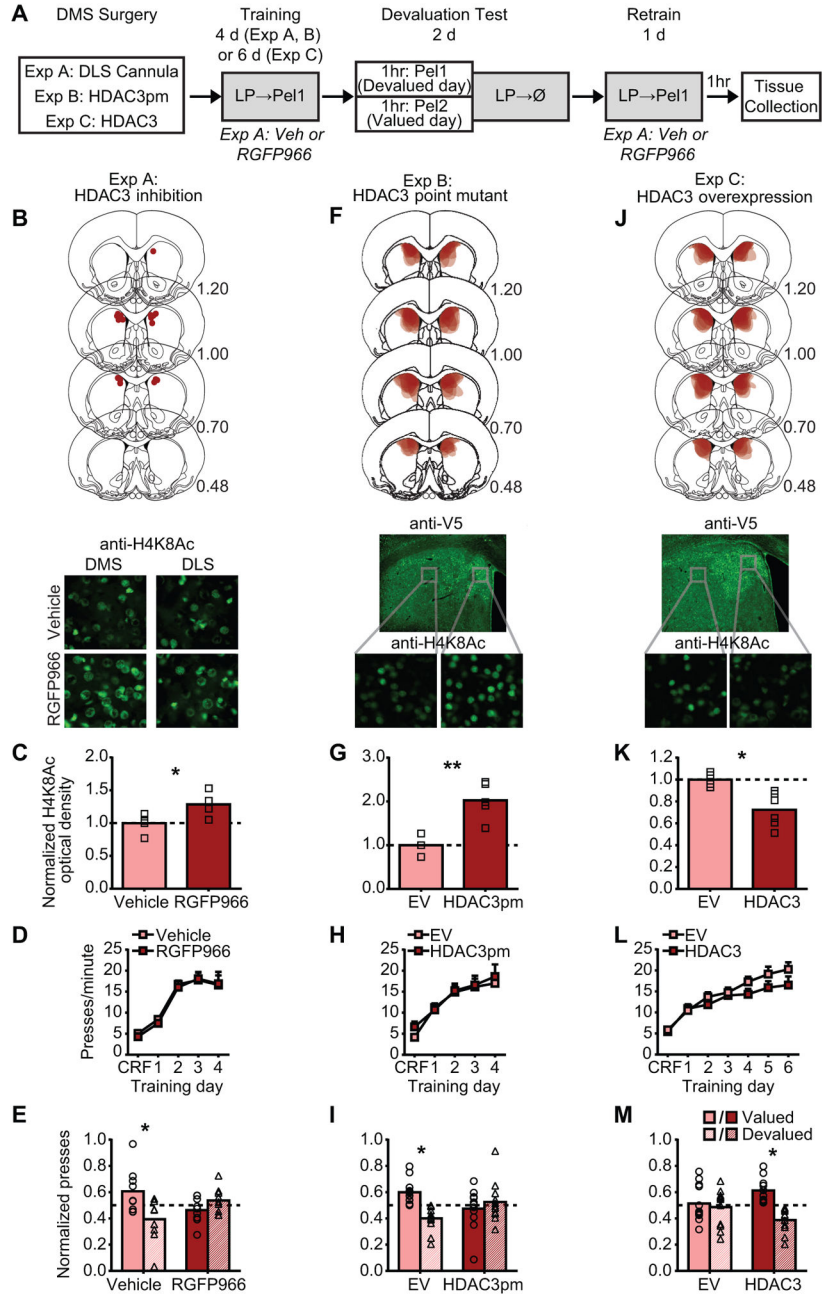
Figure 4. Effect of HDAC3 manipulation in dorsomedial striatum on habit formation

(**A**) Schematic representation of procedures. (**B**) *Top*, schematic representation of injector tips in the DMS. Numbers to the lower right of each section represent distance (mm) anterior to bregma. *Middle*, representative immunofluorescent images of H4K8Ac in DMS (*left*) and DLS (*right*) 1 hr following instrumental training/intra-DMS vehicle (*top*) or RGFP966 (*bottom*) infusion. Scale bar = 20 μm. (**F, J**) *Top*, schematic representation of HDAC3 point mutant (**F**), or HDAC3 (**J**) expression in the DMS for all subjects. *Middle*, representative immunofluorescent images of HDAC3 point mutant (**F**) or HDAC3 (**J**) expression in the DMS. *Bottom*, Representative immunofluorescent images of H4K8Ac in rats expressing the

HDAC3 point mutant (**F**) or HDAC3 (**J**) either outside (*left*) or inside (*right*) of the expression zone. (**C, G, K**) Quantification of H4K8Ac for rats receiving intra-DMS vehicle or RGFP966 infusion (**C**; N=4–5/group), intra-DMS empty vector (EV) or HDAC3 point mutant (**G**; N=4–5/group), or intra-DMS empty vector or HDAC3 overexpression (**K**; N=4–6/group). (data presented as mean + scatter). (**D, H, L**) Instrumental training performance for rats given post-training intra-DMS vehicle or RGFP966 infusions (**D**; N=9/group), intra-DMS empty vector or HDAC3 point mutant (**H**; N=11–12/group), or intra-DMS empty vector or HDAC3 (**L**; N=12/group). (data presented as mean + s.e.m). CRF (continuous reinforcement) on the first training day lever pressing was continuously reinforced with food-pellet delivery. (**E, I, M**) Normalized lever presses during the subsequent devaluation tests for rats given post-training intra-DMS vehicle or RGFP966 infusions (**E**), intra-DMS empty vector (EV) or HDAC3 point mutant (**I**), or intra-DMS empty vector (EV) or HDAC3 (**M**). *P<0.05; **P<0.01.
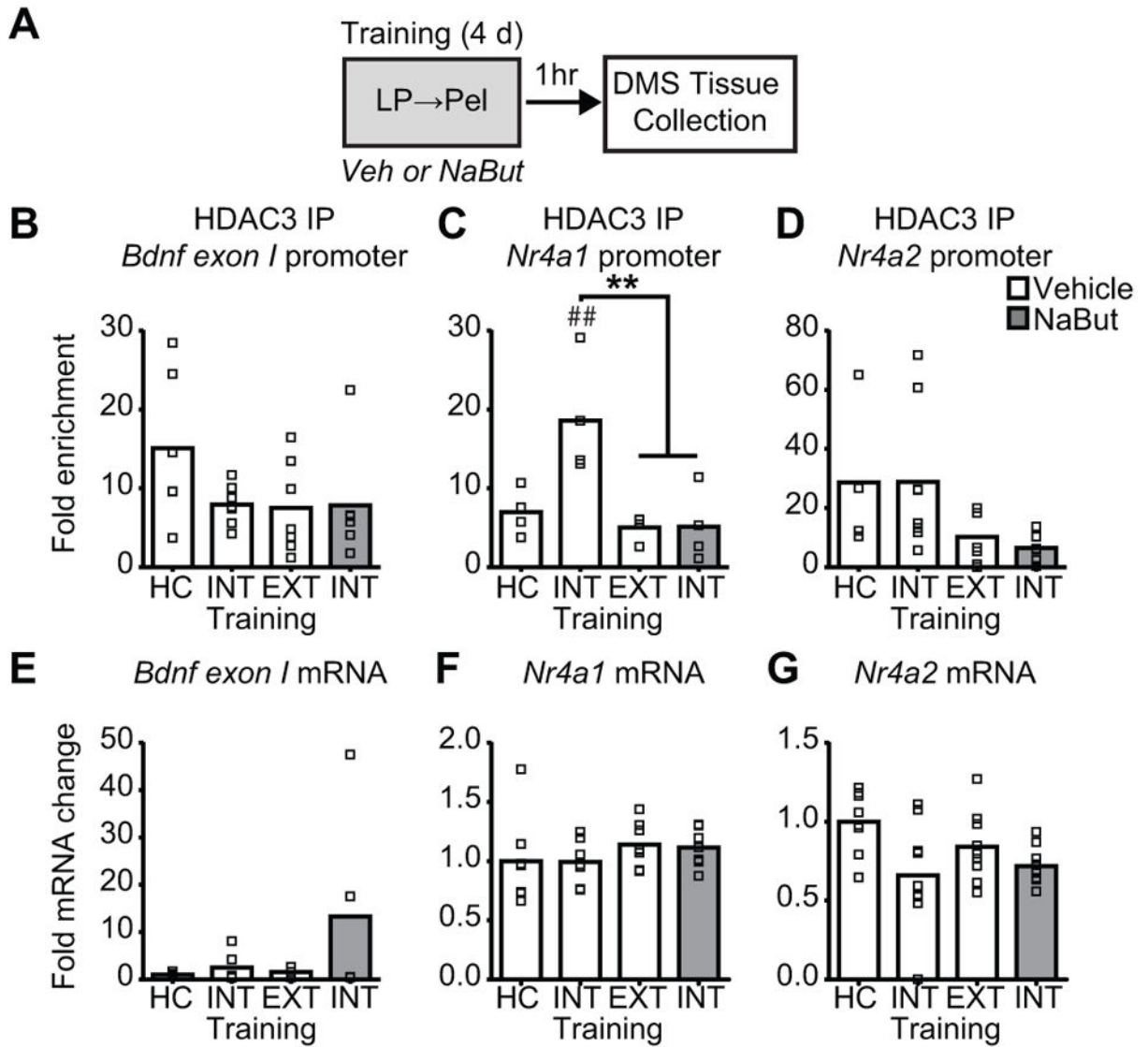
Figure 5. Effect of training and post-training HDAC inhibition on HDAC3 occupancy at learning-related gene promoters and gene expression in the dorsomedial striatum

(**A**) Schematic representation of procedures. (**B–D**) ChIP was performed with anti-HDAC3 followed by qPCR to identify HDAC3 binding to the *Bdnf1* (**B**)*, Nr4a1* (**C**)*, or Nr4a2* (**D**) promoters in the DMS of home cage (HC) controls or following either intermediate training (INT) or extended training (EXT) in vehicle-treated rats, or NaBut treatment post-intermediate training. Data presented as fold change relative to IgG (% Input/IgG (**e–g**) mRNA expression of *Bdnf1* (**E**), *Nr4a1* (**F**), and *Nr4a2* (**G**) in the DMS. **P<0.01, between groups; ##P<0.01 relative to homecage control.