Overexpressing histone deacetylase 5 in rat dorsal striatum alters reward-guided decision-making and associated neural encoding

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Manuscript information:
Number of Pages: 30
Number of words for Abstract: 227
Number of words for Introduction: 591
Number of words for Discussion: 1244
Number of words for article body and figure legends: 5113
Number of Figures: 7

Abbreviated title: HDAC5 and reward-guided decision making

Key words: histone deacetylase 5; relapse; reward-guided decision making; behavioral control; dorsal striatum; single-unit recording

CONFLICTS OF INTEREST

This research was supported by the seed grant from the Brain Behavior Initiative, University of Maryland College Park (MRR and XL), R01 DA031695 (MRR), NARSAD Young Investigator Award (XL), and R00 DA041350 (XL).

The authors declare that they do not have any conflicts of interest (financial or otherwise)

related to the data presented in this manuscript.
ABSTRACT
Accumulating evidence in the past decade implicates histone modifying enzymes, such as class I histone deacetylases (HDACs), in learning and memory, and recently habit formation. However, it is unclear whether HDACs play roles in complex cognitive function. To address this issue, we examined the role of dorsal striatal HDAC5, a class II HDAC, in reward-guided decision-making and associated neural encoding in rats. We first injected adeno-associated virus to overexpress a nuclear-localized HDAC5 in dorsal striatum (DS). We then recorded neural correlates from dorsolateral striatum (DLS) as rats performed two reward-guided choice tasks, in which we manipulated either the size of or delay to reward. During these tasks, rats first learned which of two options led to the better reward, and then reversed those contingencies in a second block of trials. We found that rats with HDAC5 overexpression in DS responded faster and chose higher value reward more often during the first block of trials, but were less able to reverse those contingencies in the second block of trials. At the neural level, HDAC5 overexpression in DS elevated and reduced the number of cells in DLS that increased firing to stimuli and reward, respectively, and shifted encoding toward cues that predicted more immediate reward. These results suggest that the HDAC5 overexpression in DS contributes to inflexible decision-making, demonstrating a role of histone modifying enzymes in complex cognitive function.

SIGNIFICANCE STATEMENT
Histone deacetylases (HDACs) are important for learning and habit formation. Here, we expanded on these functions and found that overexpression of HDAC5 produced faster and more automatic behavior, and related changes in dorsolateral striatal neural firing in rats performing a value-based decision-making task. These results implicate HDAC5 as a potential therapeutic target for psychiatric conditions that impair decision-making and executive function.
Histone deacetylases (HDACs) are a family of epigenetic enzymes that suppress gene transcription by removing acetyl groups from histone proteins (Kouzarides, 2007). Over the past decade, extensive literature has demonstrated that HDACs, primarily class I HDACs (HDAC1, 2 and 3), contribute to synaptic plasticity associated with learning and memory (Peixoto and Abel, 2013; Mahgoub and Monteggia, 2014; Penney and Tsai, 2014; Schmauss, 2017). For example, overexpressing HDAC2 in mouse hippocampus disrupts synaptogenesis, synaptic formation, long-term potentiation and hippocampal-dependent learning and memory formation, while opposite effects are observed in HDAC2-deficient mice (Guan et al., 2009). Emerging evidence recently showed that HDAC3 in rat dorsal striatum (DS) also regulates associative learning, such as habit formation (Malvaez et al., 2018). However, whether HDACs contributes to complex executive cognitive functions, such as reward-guided decision making, is unknown.

More intriguingly, how epigenetics influence neural activity at the single cell level during decision-making tasks has not been explored. Dysregulated HDAC function has been linked to aging, neurodegenerative diseases (e.g., Alzheimer's disease) and psychiatric disorders (e.g., drug addiction) (Peña et al., 2014; Hwang et al., 2017; Schmauss, 2017; Werner et al., 2021). Therefore, elucidating the role of HDACs in executive cognitive functions can help understand how dysregulated HDAC activity may lead to impaired cognitive functions under these pathological conditions (Forman et al., 2004; Volkow and Li, 2004; Koob and Volkow, 2009; Samson and Barnes, 2013; Murman, 2015; Wyss-Coray, 2016).

Here we examined the role of HDAC5, a class IIa HDAC, in rat DS in reward-guided decision making and associated neural encoding. Like other class IIa HDACs (HDAC4, 7 and 9), HDAC5 can shuttle into the nucleus from cytoplasm upon dephosphorylation in an activity dependent manner (McKinsey et al., 2000; Borrelli et al., 2008). We focused on striatal HDAC5 based on its critical role in regulating drug-related behaviors in rodent models. For example, HDAC5 and its downstream targets in nucleus accumbens (NAc) modulate the rewarding
aspect of cocaine (McKinsey et al., 2000; Renthal et al., 2007; Borrelli et al., 2008; Taniguchi et al., 2012, 2017). Work from us and others also demonstrates that HDAC5 in NAc and DS contributes to cocaine and methamphetamine relapse, respectively (Taniguchi et al., 2017; Li et al., 2018). Such findings suggest that the study of HDAC5 here can provide important insight into our observations that behavioral deficits in decision-making develop after chronic drug use (Stalnaker et al., 2006, 2009; Roesch et al., 2007; Takahashi et al., 2007; Burton et al., 2015, 2017, 2018; Brockett et al., 2018; Vázquez et al., 2019; Pribut et al., 2021).

To examine the role of HDAC5 in value-based decision-making behavior, we overexpressed HDAC5 in DS. We additionally recorded from dorsolateral striatum (DLS), a region known for its well-established involvement in forming and governing habits by encoding associative information between stimuli, outcomes and responses (e.g., S-R associations) (Yin and Knowlton, 2006; Balleine et al., 2007; Burton et al., 2015, 2017; Malvaez and Wassum, 2018). Our results showed that HDAC5 overexpression in DS promoted fast, inflexible behavior during reward-guided decision-making, accompanied by enhanced and reduced firing to cues and reward in DLS, respectively. These findings provide the first evidence, to our knowledge, that HDAC5 in DS contributes to impulsive and inflexible decision-making, behavioral deficits previously observed after chronic drug use (Stalnaker et al., 2006, 2009; Roesch et al., 2007; Takahashi et al., 2007; Burton et al., 2015, 2017, 2018; Brockett et al., 2018; Vázquez et al., 2019; Pribut et al., 2021). Therefore, HDAC5 in striatum may serve as a candidate epigenetic target that underlie the impaired cognition associated with drug addiction.

**MATERIALS AND METHODS**

**Subjects**

Eighteen Sprague-Dawley rats, both male and female, were obtained at 175-200g from Charles River Laboratories. Seven rats were excluded due to death during surgeries (n = 1) and electrode implantation issues (n = 6). The remaining 11 rats (9 males; 2 females) reported
herein refer to those used in statistical analyses. Subjects were tested at the University of Maryland, College Park in accordance with University and National Institutes of Health guidelines.

**Reward-guided choice tasks**

Prior to surgery, the rats were trained on the Delay/Size choice task (Fig. 1A) for approximately one month. During the task, rats were trained to nose poke into the central odor port upon illumination of the house light. While in the odor port, the rat was exposed to one of three odor cues (2-Octanol, Pentyl Acetate, or Carvone) that would direct how to obtain the 10% sucrose water reward. Two odor cues were forced-choices where the rat was instructed to go to either the left or right well to receive reward. The third cue was a free-choice where the rat would be rewarded at either well. The cues associated with each odor were maintained across sessions. Odors were counterbalanced across rats and presented in a pseudorandom sequence with equal distributions of left/right odors and free-choice odor occurring on 7/20 trials. Rats were water deprived to encourage motivation in the tasks. If the incorrect well was selected on a forced-choice trial, the houselights turned off and no reward was delivered. During initial training, rats were first trained to nose poke and then go to the wells for immediate delivery of reward for 1-2 days. After that, we introduced free-choice trials where rats could choose one well or the other to obtain reward. Then, we gradually introduced (2 per day) forced-choice trials. While training them on these contingencies, we progressively increased (100 ms per day) how long rats had to stay in the odor port and fluid wells, until they could remain in the odor port for 1s and wait for delayed rewards up to 7s.

Rats underwent one recording session per day and alternated between two session types: delay and size blocks. At the start of delay sessions, one well was randomly designated to have short delays (500 ms) and the other to have long delays (1000-7000 ms) prior to reward delivery. On long delay trials, the delay increased by 1000 ms each time the long delay well was...
chosen by the rat during a free-choice trial. The maximum delay time was 7000 ms and could be reduced by 1000 ms on each free choice trial if the long delay well had been chosen <8 times out of the last 10 trials to a minimum delay of 3000 ms. After 60 trials, short and long delay parameters were switched to opposite wells. At the start of size sessions, one well was randomly designated as the big well and the other as the small well. The small well delivered one 0.05 mL 10% sucrose water bolus; the big well delivered two 0.05 mL 10% sucrose water boluses, the second appearing 500 ms after the first. The delay time before reward delivery was held constant throughout the session at 500 ms. After 60 trials, the big and small parameters were switched to the opposite wells.

Adeno-associated virus (AAV) injections

We used AAV2 that shows mostly neuronal tropism (Haery et al., 2019). Both AAV2-mHDAC5 (5x10^{12} viral particles/ml) and AAV2-GFP (4x10^{12} viral particles/ml) are driven by CMV promoters. AAV2-mHDAC5 expressed a dephosphorylated mutant of HDAC5 (S259A/S279A/S498A or 3SA) that was primarily localized to the nucleus (Taniguchi et al., 2017). AAV2-GFP expressed green fluorescence proteins (GFP) and was used as the control condition. Detailed plasmid maps are available upon request. The in vivo validation of HDAC5 overexpression by AAV2-mHDAC5 and comparisons to baseline expression of HDAC5 were previously demonstrated by Li et al (Li et al., 2018).

Rats received either bilateral AAV2-mHDAC5 (n=6: 5 males and 1 female) or AAV2-GFP injections (n=5: 4 males, 1 female) as described previously (Li et al., 2018). Briefly, each hemisphere received a total of four injections (0.75 μl/injection), with two injections aiming at the dorsomedial striatum (DMS) and the other two injections aiming at DLS. We used the following coordinates from Bregma: DMS: anterior posterior(AP), +1.2 mm; medial lateral (ML), ±2.6 mm (6° angle); dorsal ventral (DV), -4.0 mm and -5.0 mm. DLS: AP, +1.2 mm; ML, ±3.8 mm (6° angle); DV, -5.0 mm and -6.0 mm. We delivered the AAVs by Hamilton syringes (32 gauge) at a
rate of 0.375 μl/min. After each injection, we left the injection needle in place for an additional minute to allow diffusion. After the final injection, we filled the drilled hole with bone wax. It is noted that we overexpressed HDAC5 in the entire DS. This is based on previous observations that HDAC5 manipulations need to be administered to the entire DS, as those targeting the DLS or DMS alone are ineffective in modulating drug seeking (Li et al., 2018).

**Electrode implantation**

Immediately after virus injections, we implanted unilateral drivable electrodes (bundles of 10-25 μm-diameter FeNiCr wires, cut at an angle so that wires are at different lengths) in the DLS (AP, +1.2 mm, ML, 3.2 mm, DV, -3.5 mm) for subsequent single-unit recordings, and we counterbalanced the hemispheres of the electrode implantations (Fig. 1C).

**Single-unit recording**

Procedures were the same as described previously (Bryden and Roesch, 2015). Wires were screened for activity daily; if no activity was detected, the rat was removed and the electrode was advanced 40 or 80 μm to reach new cells. Otherwise, a session was conducted and the electrode was advanced at the end of the session. Neural activity was recorded using four identical Plexon Multichannel Acquisition Processor systems. Signals from electrode wires were amplified 20x by an op-amp headstage, located on the electrode array. Immediately outside of the training chamber, the signals were passed through a differential preamplifier (Plexon, PBX2/16sp-r-G50/16fp-G50) where single unit signals were amplified 50x and filtered at 150-9000 Hz. Single unit signals were then sent to the Multichannel Acquisition Processor box, where they were further filtered at 250-8000 Hz, digitized at 40 kHz and amplified at 1-32x. Waveforms (>2.5:1 signal-to-noise) were extracted from active channels and recorded to disk by an associated workstation with event timestamps from the behavior computer.
Experimental Design and Statistical Analyses

Behavior during the reward-guided choice tasks were analyzed by calculating percentage choice of a particular valued condition (i.e. short, long, large, small) on free-choice trials, reaction times on free-choice trials (i.e. odor offset to odor port exit), and movement time on free-choice trials (i.e. odor port exit to fluid well entry). Like previous studies (Burton et al., 2017, 2018; Brockett et al., 2018; Vázquez et al., 2019; Pribut et al., 2021), percent choice analyses included the first and last 10 trials in their respective reward categories to examine changes in behavior after a block switch. We additionally examined behavior by session day to determine whether there were any transient changes to behavior during the recording period (Vaidya et al., 2019).

Behavioral analyses were computed for each individual session, and averaged across sessions for HDAC5 and control groups. We took the minimal number of sessions collected from one rat, and then used that same number of sessions for each rat split over the entirety of recording. Thus, each rat contributed the same number of sessions to the behavioral analyses. For reaction and movement times, we used a repeated measures analysis of variance test with factors for group (Control vs HDAC5), task (Delay vs Size), and session day as a within-subjects measure. For percent choice, we used a repeated measures ANOVA test with factors of group (Control vs HDAC5), phase (first vs last 10 trials), block (1st or 2nd), and session day as a within-subjects measure. Bonferroni corrected post hoc t-tests were used to further examine significant interaction terms.

Single units were sorted using template matching software in Offline Sorter (Plexon, Dallas, TX). Timestamps and event markers were extracted from the file using Neuroexplorer (Nex Technologies, Colorado Springs, CO). Data was analyzed using RStudio (Boston, MA) and MATLAB (MathWorks, Natick, MA). Analysis epochs were calculated by taking the total number of spikes and dividing by time. Baseline firing activity was taken 1 s before odor onset. Increasing- and decreasing-type neurons were designated based on whether firing increased or
decreased significantly relative to the baseline (Wilcoxon; p<0.05). The odor cue epoch was taken 100 ms after odor onset until well-entry. The reward epoch of 1 s encompassed 250 ms before reward delivery to 750 after reward delivery. This epoch has been used previously (Burton et al., 2017, 2018; Vázquez et al., 2019; Pribut et al., 2021) to capture firing related to the anticipation and delivery of reward. The epoch captures activity immediately preceding reward delivery without overlapping with movement-related firing even at the shortest delays (i.e., 500 ms) and captures firing related to the multiple sucrose boli delivered during large reward trials. Relationships between neural firing and behavioral activity were determined with regression tests for each neuron, separately. Specifically, regressions were performed on trial firing rates and reaction times collected during each recording session, as opposed to averaging across trials.

**Histology**

Rats were deeply anesthetized with isoflurane and perfused transcardially with 500 mL of 0.01 M phosphate buffered saline (PBS). Brain tissue was then fixed with 500 mL of 4% paraformaldehyde (PFA) for 1 h before being transferred into 30% sucrose PBS solution. Once the brains sunk, they were sectioned into 30 μm slices using a Leica cryostat and stored in cryoprotectant at -80 ºC. For HDAC5 immunohistochemistry, the sections were washed for 10 minutes in PBS and then incubated for 1 h at room temperature in blocking buffer (2% BSA in PBS with 0.3% Triton-x100). The sections were incubated next with a primary antibody against HDAC5 (sc-133106, 1:500, Santa-Cruz, TX, RRID: AB_2116793) in blocking buffer overnight at room temperature. After washing the sections 3 times in PBS (5 min each), they were incubated with the secondary antibody Alexa 594-labeled anti-mouse (R37121, 1:200, Thermo Fisher Scientific, MD, RRID: AB_2556549) in blocking buffer for 1 h at room temperature. Finally, the sections were washed in PBS and mounted on glass slides (Fisherbrand™ Superfrost™ Plus).
RESULTS

**HDAC5 overexpression in DS decreased reaction time during both delay and size tasks**

Our first analyses examined reaction time, defined as the time taken to exit the central nose port after presentation of the odor stimulus, across delay (8 days/rat) and size (6 days/rat) tasks (Fig. 2A, C). We analyzed data using a repeated measures ANOVA with factors of group (Control vs HDAC5), task (Delay vs Size), and session day. A significant main effect of task demonstrated that all rats exhibited significantly faster reaction times during size tasks ($F(1,146) = 11.005, p = 0.001$, ANOVA). Furthermore, a significant main effect of group showed that HDAC5 rats were also faster overall across both task manipulations ($F(1,146) = 4.345, p = 0.039$, ANOVA). There was no significant effect of session day ($F(1,146) < 0.01, p > 0.05$, ANOVA), nor were there any significant interactions ($F(1,146) \leq 1.734, p \geq 0.881$, ANOVA).

Based on the assumption that rats leave the odor port once they have made their choice selection, one interpretation of the reaction time result is that rats with HDAC5 overexpression are making faster decisions than control rats. However, an alternative interpretation is that HDAC5 rats simply exhibit enhanced motor responses in general. To address this issue, we also measured movement time as defined as the time from odor port exit to the fluid well as a general reflection of movement speed (Fig. 2B, D). We found no significant main effects of group ($F(1,146) = 2.426, p = 0.122$, ANOVA), task ($F(1,146) = 0.989, p = 0.322$, ANOVA), or session day ($F(1,146) < 0.01, p > 0.05$), nor were there any significant interactions ($F(1,146) \leq 2.426, p \geq 0.122$, ANOVA). Thus, HDAC5 overexpression selectively decreased reaction time but caused no general motor enhancement.

**HDAC5 overexpression in DS promoted inflexible behavior**
Decisions governed under habitual control are thought to be under the control of model-free systems that do not take into account task structures (e.g., frequent reversals), thus allowing animals to respond without deliberations and to develop associative behaviors more strongly and quickly. There is a tradeoff, however, with behavioral flexibility. In line with these theories, we found that HDAC5 rats formed stronger associations in the first block of trials that were difficult to reverse in the second block of trials.

Our next set of analyses (Fig. 2E and 2F) examined the percent choice on free-choice trials for high value choices (i.e., short-delay or large-reward), broken down into first or last 10 trials for blocks 1 and 2 (Note there were roughly 20 free-choice trials per block that were randomly interleaved with forced-choice trials). We used a repeated measures ANOVAs to analyze these data with factors of group (Control vs HDAC5), task (Delay vs Size), phase (First 10 vs. Last 10 trials within a block), block (1st vs 2nd) and session day.

For both Delay (Fig. 2E) and Size Tasks (Fig. 2F), we observed a significant main effect of phase \( (F(1,584) = 45.543, p < 0.001, \text{ANOVA}) \), indicating that all rats selected high-value rewards significantly more during the end of a trial block compared to the beginning. A main effect of block additionally showed all rats selected high-value rewards significantly more in the first compared to the second block of trials \( (F(1,584) = 35.161, p < 0.001, \text{ANOVA}) \), likely because rats were overriding previously learned reward contingencies they had acquired in the first block. There was no significant main effect of session day \( (F(1,584) < 0.01, p > 0.05, \text{ANOVA}) \) or task \( (F(1,584) = 0.004, p = 0.950, \text{ANOVA}) \). There was significant interaction between task and phase \( (F(1,584) = 5.724, p = 0.017, \text{ANOVA}) \), however Bonferroni-corrected post-hoc t-tests indicated no significant differences between either the first 10 trials of delay and size tasks \( (t(306) = -1.176, p = 0.240, \text{t-test}) \), nor the last 10 trials of delay and size tasks \( (t(306) = 2.172, p = 0.031, \text{t-test}) \). Thus, all rats generally chose more high-value rewards by the last 10 trials and during the first block, and there were no significant differences between tasks.
Interestingly, although there was no significant main effect of group (F(1,584) = 0.063, p = 0.803, ANOVA), we did observe a significant interaction between group and block (F(1,584) = 12.745, p < 0.001, ANOVA). Bonferroni-corrected post-hoc t-tests indicated that HDAC5 rats selected high-value rewards significantly more than control rats during block 1 (t(306) = 2.366, p = 0.019, t-test), but subsequently selected high-value rewards significantly less than control rats during block 2 (t(306) = -2.743, p = 0.006, t-test). All other interactions were not significant (F(1,584) < 0.001, p > 0.05). Together, these results suggest that rats generally performed best by the end of the first block and needed to reverse reward contingencies in the second block. This contrast in performance between the first and second block was significantly amplified by HDAC5 overexpression, across both delay and size tasks.

**HDAC5 overexpression in DS elevated the number of neurons that increased firing to reward predicting stimuli**

During the Delay Task, we recorded from the DLS: 485 neurons in control rats (n’s = 162, 123,111, 49, 40) and 537 neurons in HDAC5 rats (n’s = 153, 132, 118, 63, 36, 35). In control rats, 14% (n’s = 66; 31, 17, 10, 7, 1) and 39% (n’s = 189; 89, 54, 17, 15, 14) of neurons significantly increased and decreased firing during odor cue sampling, respectively (Fig. 3A; gray). In HDAC5 rats, 26% (n’s = 137; 83, 14, 14, 12, 10, 4) and 38% (n’s = 205; 88, 56, 28, 16, 9, 8) of neurons significantly increased and decreased firing, respectively (Fig. 3A; black). The frequency of increasing to decreasing neurons was significantly higher in HDAC5 rats (X² = 12.5; p = 0.0004, X²), as was the frequency of increasing to total cells recorded (X² = 14.7; p = 0.0001, X²). The counts of decreasing cells did not significantly differ between groups (X² = 0.01; p = 0.91, X²). We also found no significant difference in baseline activity in either increasing (t(171) = -0.002, p = 0.999, unpaired t-test) or decreasing cells (t(392) = 0.250, p = 0.802, unpaired t-test).
During the size task, we recorded from the DLS: 355 neurons in control rats (n’s = 103, 98, 63, 46, 45) and 478 neurons in HDAC5 rats (n’s = 143, 114, 99, 61, 33, 28). In control rats, 19% (n = 67; 38, 10, 8, 6, 5) and 29% (n = 102; 44, 26, 15, 9, 8) of neurons significantly increased and decreased firing during odor cue sampling, respectively (Fig. 3B; gray). In HDAC5 rats, 30% (n = 142; 88, 18, 13, 10, 9, 4) and 38% (n = 181; 92, 39, 27, 9, 8, 6) of neurons increased and decreased firing, respectively (Fig. 3B; black). The frequency of increasing to total cells recorded was significantly higher in HDAC5 rats ($X^2 = 7.3; p = 0.007, X^2$) than control rats, whereas the frequency of decreasing cells did not significantly differ between groups ($X^2 = 3.5; p = 0.06, X^2$). We found no significant difference in baseline activity in either increasing (t(203) = -1.273, $p = 0.204, \text{unpaired } t\text{-test}$) or decreasing cells (t(159) = 1.246, $p = 0.215, \text{unpaired } t\text{-test}$). Taken together, overexpressing HDAC5 in DS elevated the counts of DLS neurons that increased firing during sampling of reward-predicting stimuli across tasks.

**HDAC5 overexpression in DS increased the number of neurons that fired more strongly for short-delay reward**

Previously, we have shown that neurons in DLS will fire more strongly for certain directions (e.g. left or right), and for certain outcomes (e.g. short, long, big, or small) with similar distributions of each. Here, we examined temporal firing of increasing and decreasing neurons, and quantified this selectivity during cue sampling. We sorted firing into preferred (i.e. towards the neuron’s response field; Fig. 4 left panels) and non-preferred directions (i.e. away from the neuron’s response field; Fig. 4 right panels), and preferred and non-preferred outcomes, based on the direction and outcome that elicited the highest firing rate. For example, if a neuron fired the strongest to cues that predicted short delayed reward for responses made in the left direction, ‘short delayed reward’ was designated as the ‘preferred outcome’ and ‘left’ was designated as the ‘response made ‘into’ that cell’s response field. In this example ‘long delayed reward’ would be the ‘non-preferred’ outcome and ‘right’ would be the response made ‘away’
from the response field. This procedure simply allows us to average firing across all neurons, so
that we can examine temporal changes in firing to task events.

*Fig. 4A and E illustrate normalized firing – aligned to odor onset – for neurons that increased
firing in control and HDAC5 rats during delay blocks. Data were normalized by z-scoring. As
defined, increases in firing were present during odor sampling. Interestingly, while firing in
control rats peaked near the end of odor sampling (500 ms after its onset) prior to the onset of
the movement, firing in HDAC5 rats exhibited a more dramatic rise after odor presentation (> 500 ms after odor onset) during initiation of the movement, which likely reflected accelerated
responses as described above (see *Fig. 2*) and further analyzed below by showing correlations
between firing rate and reaction time at the level of single neurons.

To quantify selectivity during the delay task, we plotted the normalized difference between
short and long (delay index = short – long / short + long) for each neuron for control and HDAC5
rats. Gray bars reflect the distribution of indices across the entire population of neurons, while
black bars represent neurons that exhibit a ‘preference’ for short- or long-delay trials, firing
significantly more (above zero; short > long) or firing significantly less (below zero; long > short)
for cues that predicted short delayed reward (*Fig. 4B*). Examining activity across odor-
responsive cells from control rats, we found distributions of delay indices were not shifted
significantly above or below zero (into: *p* = 0.492, *µ* = 0.014, Wilcoxon; *Fig. 4B*, left; away: *p* =
0.616, *µ* = -0.018, Wilcoxon; *Fig. 4B*, right). Interestingly, in HDAC5 rats, the preference for
neurons to increase firing to short delayed reward increased. Unlike controls, the distribution of
delay indices in HDAC5 rats were significantly shifted above zero for movements made into to
the response field (*p* = 0.004, *µ* = 0.042, Wilcoxon; *Fig. 4F*, left), indicating that the majority of
neurons tended to fire more strongly for cues that predicted short delayed reward. Similar
results were present for neurons that decreased firing during odor presentation (i.e., *Fig. 4H*,
left; *p* = 0.045, *µ* = 0.023, Wilcoxon)
These analyses were repeated for neurons responsive to odor cues during size blocks (Fig. 5). Across conditions, response profiles were similar to those observed during delay blocks; however, unlike delay manipulations, shifts in selectivity distributions (size index; large – small/large + small) did not differ between groups, suggesting the ‘size’ encoding was not altered by HDAC5 overexpression beyond there being fewer task-related neurons overall. For increasing-type cells, none of the distributions--for either control or HDAC5 rats--were significantly shifted (Fig. 5B. into: \( p = 0.194, \mu = -0.030, \) Wilcoxon, left; away: \( p = 0.881, \mu = 0.019, \) Wilcoxon, right; Fig. 5F. into: \( p = 0.120, \mu = 0.017, \) Wilcoxon, left; away: \( p = 0.790, \mu = -0.002, \) Wilcoxon, right).

This was also observed in neurons that decreased firing for responses made into the response field (Control, Fig. 5D, left: \( p = 0.606, \mu = 0.005, \) Wilcoxon; HDAC5, Fig. 5H, left: \( p = 0.781, \mu = 0.015, \) Wilcoxon). For responses made away from the response field, distributions in both groups were shifted in the negative direction (Fig. 5D, right; \( p = 0.005, \mu = -0.041, \) Wilcoxon; Fig. 5H, right; \( p = 0.042, \mu = -0.024, \) Wilcoxon).

**Firing of single neurons was correlated with reaction time**

To better understand the relationship between behavior and changes in firing, we examined the correlation between firing rate and reaction time for each single neuron. Overall, firing tended to be negatively correlated with reaction time for movement made into a cell’s response field and positively correlated for movements made away from a cell’s response field, suggesting that increases and decreases in firing promoted and attenuated choices to be made into the cell’s response field, respectively. For movement into the cell’s response field, all distributions were shifted in the negative direction (Control delay: Fig. 6A, \( p < 0.01, \mu = -0.02, \) Wilcoxon; HDAC5 delay: Fig. 6C, \( p < 0.22, \mu = -0.01, \) Wilcoxon; Control size: Fig. 6E, \( p < 0.01, \mu = -0.02, \) Wilcoxon; HDAC5 size: Fig. 6G, \( p < 0.01, \mu = -0.02, \) Wilcoxon), whereas for movement away from the cell’s field, all distributions were shifted in the positive direction (Control delay: Fig. 6B,
**DISCUSSION**

The above analyses suggest that HDAC5 overexpression elevated firing to stimuli prior to and during the initiation of the behavioral response, and firing during the sampling of stimuli was correlated with reaction time at the single neuron level. During these analyses, we noticed that while firing was increased during presentation of odors and subsequent responding among odors during the delivery of reward appeared to be lower in HDAC5 rats than control rats (Fig. 5A and 5E). Based on this observation, we examined reward-related activity by asking how many neurons significantly increased firing during the anticipation and delivery of reward (reward epoch; $p < 0.05; \text{Wilcoxon}$). We found that 21% ($n = 102; 37, 37, 16, 7, 5$) cells in control rats increased firing to reward, whereas only 10% ($n = 54; 17, 14, 13, 4, 4$) increased responding after HDAC5 overexpression (Fig. 7A; $X^2 = 16.7, p = 4.4E-5, X^2$). However, there were no differences in baseline activity between these two populations of cells ($t(75) = -0.963, p = 0.338$, unpaired $t$-test).

The average firing of these neurons aligned to reward delivery is illustrated for both control and HDAC5 rats in Fig. 7B and C. As defined, firing increased over long delays until reward was delivered. Likewise, for size manipulations, we found 29% ($n = 104; 49, 16, 14, 13, 12$) neurons from control rats increased during the reward epoch, whereas only 10% ($n = 49; 19, 13, 7, 5, 5, 0$) were observed after HDAC5 overexpression (Fig. 7D; $X^2 = 32.4, p = 1.26E-8, X^2$). Notably, consistent with the analysis during odor sampling described above, firing of HDAC5 overexpressed ‘reward’ neurons also displayed prominent cue and movement-related firing prior to reward (Fig. 7C, HDAC5 (~3s); Fig. 7F, HDAC5 (~1s)).
Here we examined the effects of DS HDAC5 overexpression on reward-guided decision-making and associated neural correlates in DLS. Our main findings are as follows: at the behavioral level, rats with HDAC5 overexpression showed decreased reaction time and inflexible behavior on both delay and size tasks; at the neural level, we observed an increase in the numbers of neurons that fired more to reward-predicting stimuli and cues that predicted short delayed reward, but a decrease in the number of neurons that were responsive to anticipation and reward delivery following HDAC5 overexpression.

We observed a compelling increase and decrease in the selection of high-value rewards during the first and second block of trials in our HDAC5 rats. There are a number of potential explanations for this result; it is possible that learning in the first block of trials interfered with performance in the second block, or rats may have had trouble acquiring new associations once reward contingencies reversed in the second block. While it is difficult to pinpoint the exact cause of our findings, we interpret these results to be evidence of behavioral inflexibility, based on previous studies examining the roles of HDACs and dorsal striatum in habit (Malvaez and Wassum, 2018; Malvaez et al., 2018). Decisions governed under habitual control are thought to be under the control of model-free systems that do not take into account task structures (e.g., frequent reversals), thus allowing animals to respond without deliberations and to develop associative behaviors more strongly and quickly. There is a tradeoff, however, with behavioral flexibility. Here, the decrease in high-value reward selection may indicate such an inability to modify behavior once reward contingencies change.

Further support of this hypothesis may also come from the faster reaction times seen in our HDAC5 rats. In previous studies (Bryden et al., 2011; Burton et al., 2017, 2018; Brockett et al., 2018; Vázquez et al., 2019; Pribut et al., 2021) and our current study, we have used reaction time (i.e. how quickly rats leave the odor port after odor presentation) as a measure of how quickly rats decide which reward well to approach. Importantly, we observed no significant difference in movement times (i.e. how quickly rats enter the fluid well after odor port exit) in our
HDAC5 rats, indicating our reaction time findings were not just a reflection of enhanced motor output. Taken together, our behavioral findings hint a relationship between behavioral inflexibility and HDAC5 overexpression, although future studies will be needed to further explore other potential roles of HDAC5 in learning and memory mechanisms.

Most importantly, our findings suggest HDAC5 may be involved in abnormal decision-making behavior, a relationship that has thus far been largely unexplored. To date, only one other study has used a classic conditioning procedure to examine the role of HDAC3 in the formation of habitual behavior (Malvaez et al., 2018). This study, conducted by the Wassum lab, found that suppressing HDAC3 function—either through pharmacological or viral approaches, in either DLS or DMS—facilitates habit formation, while potentiating HDAC3 function through viral-mediated HDAC3 overexpression in either dorsal striatal subregions prevents habit formation.

Overall, these data indicate that, in DS, HDAC3 negatively regulates habit formation. In contrast, we showed that HDAC5 overexpression in DS led to faster inflexible behavior, implicating a positive role of HDAC5 in regulating habitual behavior.

However, direct comparison between these two studies should be made with caution. Although all HDACs generally suppress gene expression, HDAC3 and HDAC5 belong to Class I and Class IIa HDAC, respectively, and they differ in many aspects that determine their distinct functions—such as structures, the protein complexes they form, downstream targets, cellular and tissue localization, enzymatic activities and substrate specificities (De Ruijter et al., 2003; Seto and Yoshida, 2014). A question for future research is what cellular mechanisms and differential effects on the regulation of downstream targets lead to their distinct roles in habit and decision-making behavior.

Our single-unit recording data also provides the first evidence, to our knowledge, of a relationship between epigenetic mechanisms and the way in which neurons respond to different cues and reward, suggesting potential, dynamic connections amongst epigenetic events, neural activity, and changes in behaviors. With our current dataset, it is unclear whether HDAC5
overexpression produced changes in neural activity and subsequently behavior, or epigenetic changes produced behavioral changes that in turn altered patterns of neural activity within the DLS. However, we speculate that a finely orchestrated adaption of gene expression across several classes of molecules (e.g., ion channels, glutamate receptors, transcription factors), as well as alterations in intracellular signaling pathways, would be required to modify the encoding properties of neurons. These relationships will be elucidated in future studies.

An intriguing application of our data is that HDAC5 and other epigenetic enzymes may serve as a critical link between psychiatric disorders and associated cognitive impairment. For example, we and others have previously studied how drugs of abuse disrupt normal decision-making and lead to impulsive, habitual behavior (Mendez et al., 2010; Burton et al., 2017, 2018; Vázquez et al., 2019; Pribut et al., 2021). Our lab has also shown that prior cocaine experience— even long after its acute effects have worn off—altered activity within DLS. These results are remarkably similar to the current study. A robust body of work has also examined HDACs in relation to drug addiction (see Renthal and Nestler, 2009; Robison and Nestler, 2011; Rogge and Wood, 2013; Werner et al., 2021) for in-depth review). In particular, we have previously shown that dysregulated HDAC5 in DS is relevant in an animal model of methamphetamine relapse (Li et al., 2018), leading to the question of whether or not the mechanisms between relapse and drug-induced impairments in decision-making are at all shared. While addressing this question is beyond the scope of our current study, we are interested in future work where these issues are studied in tandem.

One limitation in the present study is that we overexpressed HDAC5 in both DMS and DLS; therefore, whether behavioral effects observed here require manipulations of the entire DS or specific sub-regions is unknown. However, based on our previous finding that decreased methamphetamine seeking is only observed after HDAC5 knockdown in both DMS and DLS, but not in either subregion alone (Li et al., 2018), we speculate that behavioral effects observed here require HDAC5 overexpression in the entire DS. Regarding neural correlates—although we
focused on examining DLS encoding here, we hope to include the DMS in future studies, in light of emerging evidence implicating DMS in habitual control (Stalnaker et al., 2010; Malvaez and Wassum, 2018; Vandaele et al., 2019).

In sum, we demonstrated that rats with HDAC5 overexpression in DS demonstrated inflexible behaviors and altered associated neuronal encoding in DLS. Our findings contrast with the recent examinations on the role of HDAC3 in DS in habit formation (Malvaez et al., 2018), indicating distinct mechanisms underlying decision-making and habitual control across different HDACs. Interestingly, our results were in line with previous observations of decision-making impairments after chronic cocaine use (Roesch et al., 2007; Burton et al., 2015, 2017, 2018; Brockett et al., 2018; Vázquez et al., 2019; Pribut et al., 2021). Such findings may suggest that HDACs could be a critical link between psychiatric disorders and associated cognitive impairment. Further studies will focus on the causal relationship amongst epigenetics, neural activity, and observed behavior, and how HDAC5 impacts executive control and neuronal encoding in the context of drug addiction in order to further our understanding of HDACs’ potential utility in psychiatric treatments.

Acknowledgments

We thank Dr. Christopher W. Cowan (Medical University of South Carolina) for providing us with AAV2-mHDAC5 virus.

AUTHOR CONTRIBUTIONS

MRR and XL: Conceptualization, Methodology, Formal analysis, Funding acquisition, Project administration, Resources, Supervision, Writing- Review and editing. HJP: Data curation, Formal analysis, Investigation, Writing – Original draft preparation, Writing – Review and editing. DV: Formal analysis, Investigation, Writing – Review and editing. ADW, SST, and IRD: Investigation.
CODES AND DATA ACCESSIBILITY

Raw data and Matlab codes used for behavioral and neural analyses available upon request.
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**Figure 1.** Reward-guided decision-making task, virus injections/expressions and recording sites.

(A) Schematics of the reward-guided decision-making task, showing the sequence of events in a single trial (left) and the sequence of blocks for delay and size tasks. (B) Representative images of GFP expression from control rats and HDAC5 immunostaining from HDAC5 group. White lines indicated placement of electrodes. (C) Electrode placements in control and HDAC5 rats, and virus spread in HDAC5 rats (red area in the far right diagram). Note the overlap between the recording area and virus expression in HDAC5 rats.

**Figure 2.** HDAC5 overexpression decreased reaction time and made choice behavior less flexible.

Dots represent individual rat averages. (A) Reaction time (RT; odor offset to odor port exit) averaged over all correct trials and recording sessions. We took the minimal number of sessions collected from one rat, and used that same number of sessions for each rat split over the entirety of recording for the Delay Task (control rats n’s = 162, 123, 111, 49, 40 and HDAC5 rats n’s = 153, 132, 118, 63, 36, 35). (B) Movement time (MT; odor port exit to fluid well entry). (C-D) Same as A-B during performance of the Size task (control rats n’s = 103, 98, 63, 46, 45 and HDAC5 rats n’s = 143, 114, 99, 61, 33, 28). E) Percent choice of short-delay trials during blocks 1 and 2. Within each block, trials were split into first and last 10 free-choice trials. Post-hoc t-tests for significant interactions between group and block were averaged across these early and late periods of a session. F) Same as E during performance of the Size task.

**Figure 3.** HDAC5 overexpression elevated the count of neurons that increased firing to during stimulus presentation.
Percent of cells that significantly increased or decreased firing during odor sampling (odor onset to port exit) compared to baseline (1 s epoch starting 1 s before odor onset; Wilcoxon, \( p < 0.05 \)) during the Delay (Control: 14% increased, 39% decreased; HDAC5: 26% increased, 38% decreased) (A) and Size (Control: 19% increased, 29% decreased; HDAC5: 30% increased, 38% decreased) (B) Task.

**Figure 4.** HDAC5 overexpression increased movement-related firing and the proportion of cells firing to short-delay predicting cues. (A) Average firing over all control cells that increased firing during odor sampling (increasing \( n = 66 \); decreasing \( n = 189 \)) aligned to odor onset. We sorted firing into preferred (i.e. towards the neuron’s response field) and non-preferred directions (i.e. away from the neuron’s response field), and preferred and non-preferred outcomes, based on the direction and outcome that elicited the highest firing rate. (B) Distribution of delay indices computed for neuron during the odor epoch (short-long/short+long). Gray bars reflect the distribution of indices across the entire population of neurons, while black bars represent neurons that exhibit a ‘preference’ for short- or long-delay trials, firing significantly more (above zero; short > long) or firing significantly less (below zero; long > short) for cues that predicted short delayed reward. (C,D) Same as A,B except for cells that decreased firing during the odor epoch (\( n = 189 \)). (E-H) Same as A-D with data from HDAC5 rats (increasing \( n = 137 \); decreasing \( n = 205 \)).

**Figure 5.** HDAC5 overexpression in dorsal striatum increased movement-related firing during the Size Task. (A) Average firing over all control cells that increased firing (increasing \( n = 67 \); decreasing \( n = 102 \)) during odor sampling aligned to odor onset. We sorted firing into preferred (i.e. towards the neuron’s response field) and non-preferred directions (i.e. away from the neuron’s response field), and preferred and non-preferred outcomes, based on the direction and outcome that
elicited the highest firing rate. (B) Distribution of size indices computed for neuron during the
odor epoch (large-small/ large+small). Gray bars reflect the distribution of indices across the
entire population of neurons, while black bars represent cells with significant differences
between large and small (Wilcoxon; $p < 0.05$). (C,D) Same as A,B except for cells that
decreased firing during the odor epoch ($n = 102$). (E-H) Same as A-D with data from HDAC5
rats (increasing $n = 142$; decreasing $n = 181$).

**Figure 6. Firing in dorsolateral striatum was correlated with reaction time.**

(A-D) Distribution of R values for within cell correlations between firing rate and reaction time in
control (A,B) and HDAC5 (C,D) rats for movements made into (A,C; left panels) and away from
(B,D; right panels) from the response field.

**Figure 7. HDAC5 overexpression reduced counts of neurons that increased firing to
reward delivery.**

(A) Percentage of neurons that increased firing during the reward epoch ($1 \text{ s; 250 ms before to}$
$750 \text{ ms after reward delivery}$). (A,C) Average firing of neurons that increased firing during the
reward epoch in control ($n = 102, 21\%$) and HDAC5 rats ($n = 54, 10\%$) in the Delay Task. Firing
is aligned to reward delivery. (D-F) Same as A-C with data from the Size Task (control = 104,$
29\%$; HDAC5 = 49, 10%).
Figure 1

A

Delay Task or Size Task

Block 1
Bias Left
Short-left

Block 2
Bias Right
Long-left

Block 1
Bias Left
Big-left

Block 2
Bias Right
Small-left

B

GFP

HDAC5

C

Control

HDAC5

~+1.2 mm
Figure 2

A
Free Choice RT - Delay

B
Free Choice MT - Delay

C
Free Choice RT - Size

D
Free Choice MT - Size

E
Delay Choice

F
Size Choice
Figure 3

A. Delay

- Control
- HDAC5

Percent of Significant Neurons

B. Size

- Control
- HDAC5

Percent of Significant Neurons
Figure 4

Control Increasing - Delay Task

A. Into the Response Field

Preferred Outcome
Non-Pref Outcome

B. Long > Short : Short > Long

p = 0.492
µ = 0.014

C. Into the Response Field

Preferred Outcome
Non-Pref Outcome

D. Long > Short : Short > Long

p = 0.790
µ = 0.004

HDAC5 Increasing - Delay Task

E. Into the Response Field

Afay from Response Field

F. Long > Short : Short > Long

p = 0.004
µ = 0.042

Control Decreasing - Delay Task

G. Into the Response Field

Preferred Outcome
Non-Pref Outcome

H. Long > Short : Short > Long

p = 0.045
µ = 0.023

HDAC5 Decreasing - Delay Task

G. Into the Response Field

Preferred Outcome
Non-Pref Outcome

H. Long > Short : Short > Long

p = 0.981
µ = -0.018

p = 0.071
µ = 0.024

p = 0.616
µ = -0.018

p = 0.505
µ = -0.013

p = 0.045
µ = 0.023

p = 0.981
µ = -0.018
Figure 5

**Control Increasing - Size Task**

A. Into the Response Field

B. Small > Big, Big > Small

**Control Decreasing - Size Task**

C. Into the Response Field

D. Small > Big, Big > Small

**HDAC5 Increasing - Size Task**

E. Into the Response Field

F. Small > Big, Big > Small

**HDAC5 Decreasing - Size Task**

G. Into the Response Field

H. Small > Big, Big > Small

| Time from Odor Onset (s) | Count | Big-Small / Big+Small (spikes/sec) |
|-------------------------|-------|-----------------------------------|
| 30                      | 15    | Big-Small / Big+Small              |
| 60                      | 30    | Big-Small / Big+Small              |
| 40                      | 40    | Big-Small / Big+Small              |
| 80                      | 60    | Big-Small / Big+Small              |

- $p = 0.194$, $\mu = -0.030$
- $p = 0.881$, $\mu = 0.019$
- $p = 0.120$, $\mu = 0.017$
- $p = 0.790$, $\mu = -0.002$
- $p = 0.606$, $\mu = 0.005$
- $p = 0.005$, $\mu = -0.041$
- $p = 0.761$, $\mu = 0.015$
- $p = 0.042$, $\mu = -0.024$

The plots illustrate the normalized firing rate with time from odor onset, showing the statistical significance ($p$-value) and mean ($\mu$) for different scenarios.
Figure 6

- **Delay Task**
  - **A** Into the Response Field: Control $p < 0.01$, $\mu = -0.02$
  - **B** Away from Response Field: Control $p < 0.01$, $\mu = 0.02$
  - **C** Into the Response Field: HDAC5 $p = 0.22$, $\mu = -0.01$
  - **D** Away from Response Field: HDAC5 $p < 0.01$, $\mu = 0.03$

- **Size Task**
  - **E** Into the Response Field: Control $p < 0.01$, $\mu = -0.02$
  - **F** Away from Response Field: Control $p < 0.01$, $\mu = 0.04$
  - **G** Into the Response Field: HDAC5 $p < 0.01$, $\mu = -0.02$
  - **H** Away from Response Field: HDAC5 $p < 0.01$, $\mu = 0.02$
Figure 7

A. Delay Task

B. Short Long

C. Short Long

D. Size Task

E. Big Small

F. Big Small

Normalized Firing Rate

Time from Reward (s)