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Histone deacetylase 1 regulates haloperidol-induced motor side effects in aged mice

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Abstract

Background: Antipsychotic drugs prescribed to elderly patients with neuropsychiatric disorders often experience severe extrapyramidal side effects. Previous studies from our group suggest that changes in histone modifications during aging increase the risk for antipsychotic drug side effects, because co-administration of antipsychotics with class 1 histone deacetylase (HDAC) inhibitors could mitigate the severity of motor side effects in aged mice. However, which HDAC subtype contributes to the age-related sensitivity to antipsychotic drug side effects is unknown.

Methods: In this study, we overexpressed histone deacetylase type 1(HDAC1) in the striatum of 3-month-old mice and knocked down HDAC 1 in the striatum of 21-month-old mice by microinjection of AAV9-HDAC1-GFP or AAV9-CRISPR/Cas9-HDAC1-GFP vectors. Four weeks after the viral-vector delivery, the typical antipsychotic drug haloperidol was administered daily for 14 days, followed by motor function assessments through the open field, rotarod, and catalepsy behavioral tests.

Results: Young mice with overexpressed HDAC1 showed increased cataleptic behavior induced by haloperidol administration, which is associated with the increased HDAC1 level in the striatum. In contrast, aged mice with HDAC1 knocked down rescued locomotor activity, motor coordination, and decreased cataleptic behavior induced by haloperidol administration, which is associated with decreased HDAC1 level in the striatum.

Conclusions: Our results suggest that HDAC1 is a critical regulator in haloperidol-induced severe motor side effects in aged mice. Repression of HDAC1 expression in the striatum of aged mice could mitigate typical antipsychotic drug-induced motor side effects.

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CRediT authorship contribution statement

Bryan McClarty: Methodology, Investigation Data curation, Visualization, Writing – original draft preparation. **Saikat Chakraborty:** Conceptualization, Methodology, Data curation, Investigation, Writing – review & editing. **Guadalupe Rodriguez:** Methodology, Investigation, Data curation, Writing – review & editing. **Hongxin Dong**: Supervision, Writing – review & editing.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bbr.2023.114420.

Keywords

Gene editing; Histone deacetylase 1 (HDAC1); Antipsychotics; Motor side effects; Mice

1. Introduction

Antipsychotic drugs are commonly prescribed not only to elderly patients with psychiatric disorders but also to dementia patients with severe neuropsychiatric symptoms, including agitation, aggression, disinhibition, depression, hallucinations and paranoia [1–4]. However, severe side effects, particularly motor side effect, often happens in these patients [5–7]. Two main classes of antipsychotic drugs currently used in clinical practice are typical and atypical antipsychotics. Typical antipsychotics are the first generation of antipsychotic drugs with the function of a dopamine receptor 2 (D2R) antagonist; however, due to the high affinity of binding on D2R, the likelihood of extrapyramidal side effects (EPS) is increased. EPS symptoms include dystonia, akathisia, parkinsonism, bradykinesia, tremor, and tardive dyskinesia resulting from extrapyramidal system dysfunctions.

Conversely, atypical antipsychotic drugs target D2R and 5-HT2A through antagonism, and the binding affinity of atypical antipsychotics to D2R is relatively weak. Therefore, the likelihood of EPS is lower. However, in aged patients, atypical antipsychotics still could induce EPS [8–10].

The mechanisms underlying EPS due to excessive antagonism of D2R in the extrapyramidal system, particularly the nigrostriatal pathway, leading to reduced receptor output [11–13]. Moreover, human studies showed that D2R availability and functionality could influence typical antipsychotic drug-induced EPS [14,15]. Additionally, studies in animal models from our group showed that age-related decreases in D2R protein expression might affect typical antipsychotic drug motor side effects [16,17]. However, the mechanisms linked to the dynamic changes of D2R expression during aging still need further investigation.

Histone modifications, particularly histone acetylation, significantly regulate brain function and aging. Dysregulation of histone acetylation is associated with many neurological disorders including Alzheimer’s disease [18–23]. Histone acetylation is controlled by two enzymes: histone deacetylases (HDACs) and histone acetyltransferases (HATs). HDACs are responsible for histone deacetylation, which represses gene transcription. Conversely, HATs are responsible for histone acetylation, which promotes gene transcription. HDACs are divided into five classes based on homology, function, and location within the cells [24,25]. HDACs have a wide range of roles in various biological processes in the organisms [24]. HDACs have been shown to regulate memory, cellular senescence, and inflammation in the brain. [16,26–32]. Studies show that administering class 1 HDAC inhibitors improves memory function by restoring histone acetylation in animal models [16,28,33–38]. In our previous work, we also found that HDAC inhibitors could improve motor and memory function by rescuing histone acetylation at the *Drd2* promoter and restoring D2R expression in the striatum of aged mice after combined administration of selective class 1 HDAC inhibitors VPA, MS-275, and CI-994 with haloperidol, suggesting that class 1 HDACs are essential regulators for antipsychotic drug activity through effects of histone acetylation at

the target receptor gene promotor [16,17]. However, our previous studies cannot dissect which subtype of HDACs plays a critical role in the regulation of antipsychotic action due to the non-specificity of the HDAC inhibitors. In this study, we overexpressed HDAC1 by AAV9-HDAC1-GFP delivery into the striatum of 3-month-old mice, and knocked down HDAC1 by AAV9-CRISPR/Cas9-HDAC1-GFP delivery into the striatum of 21-month-old mice, to investigate the dynamic changes of HDAC1 during aging and the impact on antipsychotic-induced motor side effects. Our results showed that overexpression of HDAC1 increased cataleptic behaviors after haloperidol administration in young mice, which is associated with increased HDAC1 expression in the striatum. Conversely, HDAC1 knock-down decreased haloperidol-induced motor side effects, including restored locomotor activity and motor coordination, and decreased severity of catalepsy in aged mice, which is associated with decreased HDAC1 level in the striatum. Our results confirmed that HDAC1 is vital in regulating antipsychotic action.

2. Materials and methods

2.1. Mice

Young (2–3 months old, total n = 32) and aged (21 months old, total n = 32) C57BL/6 male and female mice were equally distributed in each experimental group. Four independent groups per age are applied (young mice: AAV9-GFP+VEH, AAV9-GFP+HAL, AAV9-HDAC1+VEH, and AAV9-HDAC1+HAL; aged mice: sgRNA+VEH, sgRNA+HAL, CRISPR-HDAC1+VEH and CRISPR-HDAC1+HAL, n = 8 for behavioral tests, n = 3 per group for biochemical analysis) from Charles River laboratories were used for this study. Animals were group-housed on a 12-h light/dark cycle and given food and water ad libitum. All procedures were performed according to NIH guidelines for treating animal subjects and the Current Guide for the Care and Use of Laboratory Animals (2011, eighth edition) under a protocol approved by the Northwestern University Animal Care and Use Committee.

2.2. Viral delivery

Viral preparation: For our overexpression studies, Vector BioLabs® packaged AAV9-HDAC1-GFP (AAV9-HDAC1) viral vectors containing the mouse HDAC1 sequence to achieve overexpression. Control vectors AAV9-GFP (AAV9-GFP) without HDAC1 were provided. For our knockdown studies, ABM technologies® packaged AAV9-CRISPR/Cas9-HDAC1 (CRISPR-HDAC1) viral vectors to knock down HDAC1. In addition, scramble sgRNA control vectors AAV9-scramble sgRNA (scramble sgRNA) were provided. A total of 1.0 μL of each viral vector at 10 e9 GC/ml titer was injected bilaterally into the striatum. To achieve target cell (neuron) specific gene expression in the striatum, we used AAV9 vectors with the mouse synapsin promotor [39]. Viral injection: The mice were anesthetized and then positioned in a Kopf stereotaxic frame with a 10 μL Hamilton microsyringe (Hamilton Co., Reno, NV, USA), filling the vector fitted with a steel cannula. Injections into the striatum were made at the following stereotaxic coordinates: 0.26 mm rostral to bregma, 2.3 mm lateral to the midline, and 3.7 mm ventral to the dura. The microinjections were carried out at a rate of 4 nL/second for a total volume of 1.0 μL virus in 15 min. After injections, the cannula remained in situ for an additional 15 min before being withdrawn.

3. Drugs

The antipsychotic drug haloperidol (HAL) was purchased from Sigma (St. Louis, MO). Haloperidol (0.05 mg/kg) was first dissolved in 50 μL of glacial acetic acid and brought to the final dose volume in 0.9% saline with pH adjusted to 5–6 measurement titrated with 0.1 M NaOH. We selected haloperidol at a dose of 0.05 mg/kg for this study because our previous work showed that a 0.01–0.1 mg/kg range could induce EPS-like behaviors in aged mice [17]. Haloperidol was prepared freshly on the day of administration. All compounds and vehicles were administered intraperitoneally at a constant volume of 10 μL/g of body weight once a day for 14 consecutive days (Fig. 1). Immediately after drug administration, we carefully monitored animal physical condition and behaviors, including respiratory stress, locomotor function, and body weight. Respiratory distress was determined by breathing patterns in mice. If fast/short, labored breathing was observed for more than 30 min after injections, and this symptom persisted 1-day post injections it was marked as respiratory distress. General locomotor activity was monitored by observation 5, 10, and 20 min after the first day of injections. Then we measured locomotor activity 30 min after the first day of injections using the open-field test. Locomotor function impairments were considered if a mouse's movement was significantly reduced or had no movement compared to control mice more than 30 min after drug administration. Body weight was measured daily, and any weight loss (>2 g) within 2–3 days was considered a significant side effect.

4. Behavioral tests

Mice were acclimated to a soundproof behavioral testing room 30 min before testing, and assays were performed during the light part of the 12-h light/dark cycle. Motor function tests were conducted during the second week of drug administration (Fig. 1). The purpose of our experimental design was trying to reveal the maximum drug effects in 14 days, including a long-term (14 days) and short-term (30–60 min) results of the antipsychotic drug on motor behavior. Similar experimental designs have been reported in previous studies in our group or other fields [17,40–43]. The order of the behavioral test was as follows: open field, rotarod, and catalepsy (Fig. 1). The behavioral tests and data analysis were conducted by one investigator blinded to viral vector and treatment conditions.

4.1. Open field

The apparatus consisted of an evenly illuminated plexiglass box (40 cm × 40 cm × 40 cm) placed on a stable table with overhead video recording. Locomotor activity was defined as the distance traveled (m) during a 10 min [44]. Animal activity was recorded using an automated tracking system (Any-Maze, Stoelting, Wood Dale, IL).

4.1.1. Catalepsy—The Step-Down assay was used for cataleptic behavior. Briefly, a plastic rod (1 cm diameter) was suspended 3.5 cm above a laboratory bench in a soundproof behavioral room. Thirty minutes after the drug injection, the animal's front paws were placed on the rod while the hind feet rested on the bench. The duration of a cataleptic episode was defined as the time to step off from the rod during a 300-seconds trial [17,45].

4.1.2. Motor coordination—The TSE Rotarod System (Bad, Homburg, Germany) was used to assess motor coordination after drug administration. Mice were placed on an accelerating rod (4–40 rpm during the first 5 min) for 10 min, and the latency to fall from the rod was recorded. A total of 4 trials were conducted with a 10-min inter-trial interval. The average latency to fall from the rod across the four trials was calculated and used for comparison [17,46].

4.2. Biochemical analysis

4.2.1. Western blot—After completion of behavioral tests, we first collected brain tissues through cardiac perfusion with 1X PBS solution for 1 min to wash out blood from blood vessels in the mouse brain. The brains were then collected and quickly dissected on an ice-cold Preti dish using a dissecting scope. The striatum and prefrontal cortex were frozen at − 80 °C until ready for processing for molecular analysis. The abundance of HDAC1 was determined in the lysates of the striatum. Protein extraction was performed by homogenizing approximately 40 mg of tissue in a mix of ice-cold RIPA buffer (catalog # R0278, Sigma-Aldrich, St. Louis, MO) and protease inhibitor cocktail solution (catalog # PI78410, Fisher Scientific, Hampton, NH). Tissues were processed first by using a cordless motor connected to a Teflon pestle (20 s; catalog # 12–141–362, Fisher Scientific, Hampton, NH) followed by sonication with Branson 450 Digital Sonifier (amplitude 70%, 2–3 s; catalog # B450, Marshall Scientific, Hampton, NH). Samples were centrifuged at 20,000 g for 10 min at 4 °C, and supernatants were collected to determine total protein concentration. Protein content was measured using the Pierce™ BCA protein assay kit (catalog # PIA53226, Fisher Scientific, Hampton, NH, 2019). An equal amount of proteins (20 μg) were loaded and resolved through electrophoresis in 10% Criterion™ TGX Stain-Free™ Precast Gels at 100 V for 1.5 h (catalog # 5671035, Biorad, Hercules, CA, 2019). Proteins were transferred onto a polyvinylidene difluoride membrane. Blots were blocked with a 5% non-fat dry milk solution for 1 h at room temperature and immunostained overnight at 4 °C with primary antibodies at 1:1000 dilution against HDAC1 (Abcam, catalog # ab109411, rabbit) and β-actin (Santa Cruz, catalog # sc-47778, mouse). The next day, membranes were incubated with 1:10,000 dilution of goat anti-mouse IgG (H+L) cross-adsorbed secondary antibody, Alexa Fluor 700 (Invitrogen A-21036), and goat anti-rabbit IgG (H+L) cross-adsorbed secondary antibody, Alexa Fluor 800 (Invitrogen A32735). Immunoblots were imaged using the Odyssey® CLx system (Licor) and Image J Software (Fiji). The levels of target protein expression were normalized to β-actin.

5. Statistical analysis

All statistical analyses were conducted using the GraphPad prism software (San Diego, CA). Data are expressed as mean ± standard error of the mean (SEM). Two-way analysis of variance (ANOVA) followed by a multiple comparison analysis using Tukey’s post hoc method was used to detect drug and viral vector effects on behavior changes. Additionally, an unpaired t-test was used to verify HDAC1 overexpression and knockdown for the drug effects on behavior changes.

6. Results

6.1. Confirmation of genetic overexpression of HDAC1 in young mice and knockdown of HDAC1 in aged mice

For HDAC1 over expression in young mice, AAV9-HDAC1 was delivered in the striatum in 3-month-old mice. After four weeks AAV9-HDAC1 transfection, young mice displayed a significant increase of HDAC 1 protein expression compared to AAV9-GFP controls ($p = 0.0110$, Fig. 2A, B). For knockdown of HDAC1 expression in aged mice, CRISPR-HDAC1 was delivered in the striatum of 21-month-old mice. After four weeks of CRISPR-HDAC1 transfection, aged mice displayed a significant decrease of HDAC1 protein expression in the striatum compared to scramble sgRNA controls ($p = 0.0063$, Fig. 2C, D).

6.2. HDAC1 overexpression in the striatum impacts the haloperidol-induced motor side effects in young mice

We wanted to know whether overexpression of HDAC1 in the striatum of young mice could exacerbate motor side effects induced by haloperidol, commonly seen in aged mice. We first measured general locomotor activity through the open field test. Two-way ANOVA revealed a significant effect of the drug ($F_{1,28} = 28.01$, $p < 0.0001$) and a trend effect of AAV9-HDAC 1 ($F_{1,28} = 3.092$, $p = 0.0896$), but no drug and AAV9-HDAC1 interaction on the total distance traveled in the open field test (Fig. 3A). We then conducted a t-test analysis to compare the vehicle and haloperidol treatment regardless of viral groups, and found that mice receiving chronic haloperidol treatment had decreased general locomotor activity in the open field test ($p < 0.0001$, supplementary Fig. 1A).

Next, we measured motor coordination through the rotarod behavior test. Two-way ANOVA revealed a significant effect of the drug ($F_{1,28} = 20.80$, $p < 0.0001$) and AAV-HDAC1 ($F_{1,28} = 7.926$, $p = 0.0088$), but no drug and AAV-HDAC1 interaction on the latency to fall from the rod open field test (Fig. 3B). Again, we conducted a t-test analysis on drug effect regardless of viral groups and found that mice receiving chronic haloperidol treatment had decreased motor coordination in the rotarod test ($p = 0.0002$, supplementary Fig. 1B). Furthermore, additional t-test analysis on the virus effect regardless drug group and found that mice who received AAV9-HDAC1 transfection had decreased motor coordination in the rotarod test ($p = 0.0352$, supplementary Fig. 1C).

Lastly, we measured muscle rigidity through the catalepsy test (Fig. 3C). Two-way ANOVA revealed significant effects of the drug ($F_{1,28} = 240.2$, $p < 0.0001$) and AAV-HDAC1 ($F_{1,28} = 35.11$, $p < 0.0001$), and drug x AAV-HDAC1 interaction ($F_{1,28} = 38.57$, $p < 0.0001$) on the duration of cataleptic episodes. *Post-hoc* analysis revealed a significant increase in cataleptic episodes in AAV9-GFP+HAL and AAV9-HDAC1+HAL treated mice as compared to AAV9-GFP+VEH and AAV9-HDAC1+VEH treated mice (both $p < 0.0001$ respectively); however, AAV9-HDAC1+HAL treated mice increased cataleptic duration compared to AAV9-GFP+HAL treated mice ($p < 0.0001$).

6.3. HDAC1 knockdown in the striatum impact on haloperidol-induced motor side effects in aged mice

We also wanted to know whether the knockdown of HDAC1 expression in the striatum of aged mice could reduce haloperidol-induced motor side effects. We first measured general locomotor activity through the open field test. Two-way ANOVA revealed significant effects of the drug ($F_{1,32} = 43.38$, $p < 0.0001$) and CRISPR-HDAC1 ($F_{1,32} = 8.042$, $p = 0.0079$), and drug and CRISPR-HDAC1 interaction ($F_{1,32} = 10.08$, $p = 0.0033$) on the total distance traveled in the open field test (Fig. 4A). *Post-hoc* analysis showed a significant decrease in locomotor activity in scramble sgRNA+HAL treated mice as compared to scramble sgRNA+VEH and CRISPR-HDAC1+VEH (both $p < 0.0001$, respectively), However, CRISPR-HDAC1 +HAL treated mice displayed significant improvement in locomotor activity ($p < 0.0009$).

Next, we measured motor coordination through the rotarod test. Two-way ANOVA revealed significant effects of the drug ($F_{1,32} = 77.06$, $p < 0.0001$) and CRISPR-HDAC1 ($F_{1,32} = 6.370$, $p = 0.0168$), and a drug and CRISPR-HDAC1 interaction ($F_{1,32} = 6.145$, $p = 0.0186$) on the latency to fall from the rotarod (Fig. 4B). *Post-hoc* analysis revealed scramble sgRNA+HAL and CRISPR-HDAC1+HAL treated mice showed a decrease in motor coordination compared to scramble sgRNA+VEH ($p < 0.0001$) and CRISPR-HDAC1+VEH ($p = 0.0003$) treated mice,. However, CRISPR-HDAC1+HAL treated mice displayed a better motor coordination as compared to scramble sgRNA +HAL treated mice ($p = 0.0075$).

Lastly, we measured muscle rigidity through the catalepsy behavior test. Two-way ANOVA revealed significant effects of the drug ($F_{1,32} = 167.3$, $p < 0.0001$) and CRISPR-HDAC1 ($F_{1,32} = 26.55$, $p < 0.0001$), and drug and CRISPR-HDAC1 interaction ($F_{1,32} = 20.71$, $p < 0.0001$) on the duration of cataleptic episodes (Fig. 4C). *Post-hoc* analysis revealed a significant increase in cataleptic episodes in scramble sgRNA+HAL and CRISPR-HDAC1 +HAL treated mice compared to scramble sgRNA+VEH and CRISPR-HDAC1+VEH treated mice (both $p < 0.0001$, respectively). However, CRISPR-HDAC1 +HAL treated mice showed a significantly decreased cataleptic duration compared to scramble sgRNA+HAL treated mice ($p < 0.0001$).

7. Discussion

In this study, we used two distinct gene editing approaches (AAV-HDAC1 and CRISPR-HDAC1) to investigate how changes in HDAC1 expression in young or aged mice impact typical antipsychotic drug haloperidol induced motor side effects. Specifically, we overexpressed HDAC1 in the striatum of young mice through AAV-HDAC1 transfection resulting in accelerated cataleptic episodes after chronic haloperidol administration. In contrast, we knocked down HDAC1 in the striatum of aged mice through CRISPR-HDAC1 transfection resulting in the improvement of general locomotor activity and motor coordination, and mitigation of cataleptic episodes induced by chronic haloperidol administration. Together, the results from our study confirmed that HDAC1 plays a vital role in regulating antipsychotic action in aged mice.

We selected haloperidol, a classic typical antipsychotic, testing in the genetic HDAC1 manipulated mouse models to understand the role of HDAC1 on haloperidol-induced side effects as haloperidol has been shown to increase age-related sensitivity of the motor side effects and cause severe EPS in both clinical and preclinical studies [16,17,47–53]. The mechanisms underlying the age-related sensitivity to haloperidol-induced side effects have yet to be well understood. Our previous studies suggest a potential epigenetic mechanism regulating haloperidol-induced side effects through the primary target, D2R [16, 17]. Specifically, we found a decrease in histone acetylation at the *Drd2* gene promoter, followed by reductions in D2R protein expression in the aged striatum. Additionally, chronically co-administered class 1 HDAC inhibitors with haloperidol could improve motor function, restore acetylation at *Drd2,* and increase D2R protein expression in the striatum of aged mice [16,17]. However, the therapeutic agents of the HDAC inhibition we used in these studies are either boarding inhibition of class 1 HDACs or inhibition of HDAC 1, 2, and 3. The selective individual class 1 HDAC subtype inhibitors are still unavailable. Moreover, given that HDAC inhibitors themselves could induce side effects, it is essential to dissect which subtype of HDACs plays a critical role in regulating antipsychotic action to help development of specific agents that are most effective in mitigating antipsychotic-induced side effects.

Based on our previous results, in this study, we focused on HDAC1. We found that transfection of AAV-HDAC1 into the striatum of young mice could induce overexpression of HDAC1 and result in more severe cataleptic behaviors after haloperidol administration. More interestingly, we found that HDAC1 overexpression significantly decreased motor coordination, although the interaction of drug and AAV-HDAC1 was not found. Our previous studies have shown that milder motor side effects induced by haloperidol in young mice and class 1 HDACs inhibitors could decrease such side effects including motor coordination impairment and catalepsy [16,17,49]. Currently, there are no studies aside from our own reported an increase in HDAC1 itself could impact motor function. However, future work is needed to confirm this effect.

Then, we transfected CRISPR-HDAC1 into the striatum in the aged mice, successfully knocked down HDAC1 expression and sufficiently decreased haloperidol-induced motor side effects. These results align well with our previous findings with HDACs inhibitor and provide convincing evidence indicating that HDAC1 is vital in regulating antipsychiatry drug actions. Our previous studies found a decrease of histone acetylation at the *Drd2* gene promoter in the striatum of aged mice, and chronically co-administered class 1 histone deacetylase inhibitors (VPA and MS275), which have targeted class 1 HDACs, which includes HDAC1, with haloperidol could improve motor function, restored acetylation at *Drd2*, and increased D2R protein expression in aged mice [16,17]. Our current results of CRISPR-HDAC1 knocked down in aged mice, further support the hypothesis that HDAC1 is an important modulator in haloperidol-induced motor deficits, which is likely causing histone hypoacetylation at *Drd2* gene promotor resulting in the decreased D2R expression and functionality. We propose that transfected CRISPR-HDAC1 in the striatum could decrease HDAC1 blockage on the *Drd2* gene promotor, increase D2R protein expression. However, this proposed molecular mechanism needs to be further confirmed in future

studies by investigating HDAC1 abundance at the *Drd2* gene promoter, histone acetylation changes at *Drd2*, and D2R expression after HDAC1 modification in young or aged mice.

HDAC1 is a crucial regulator for histone acetylation in the brain; we expect such mechanism could happen after overexpression or knockdown of HDAC1 in young or aged mice. Our previous work indicates that HDACs are differently expressed in the striatum of young and aged mice, and this difference may contribute to the response differences of young and aged mice to the antipsychotic drug [16,17]. Increased HDACs expressions in aged mice were associated with decreased histone acetylation at *Drd2*, resulting in a decrease in D2R expression and function. We propose that such mechanisms are also partially applied in young mice when we genetically increase the HDAC1 expression in the striatum to repress the *Drd2* gene expression, resulting in increased sensitivity in response to haloperidol-induced cataleptic behavior. However, in young mice, other epigenetic factors may stay normal and could have a compensatory influence on such effects.

We must mention that in addition to motor side effects, haloperidol could induce other side effects, including memory impairment [54–56]. Histone acetylation has also been shown to be associated with age-related memory changes in mice; therefore, understanding the regulation of HDAC1 in histone acetylation changes associated with antipsychotic-induced memory deficits is also necessary. It is unknown whether genetic editing by overexpression of HDAC1 in young mice and knock-down of HDAC1 in aged mice could influence other HDACs in class 1, such as HDAC2 or HDAC3 expression, as a potential compensatory effect of other HDACs could happen after genetic manipulation [57–59]. Our future work will include the confirmation of HDAC2 and 3 expressions in the striatum to rule out potential compensatory effects. It is also necessary to genetically edit HDAC2 or 3 in the striatum to compare and determine the individual contribution of class 1 HDACs to antipsychotic drug action.

In summary, the results of this study suggest that HDAC 1 is an essential regulator in typical antipsychotic drug-induced motor side effects. It is likely that increased HDAC 1 in aging is driving histone hypoacetylation at the *Drd2* gene promoter, reducing receptor output and increasing EPS-like behaviors. Developing a more selective HDAC 1 inhibitor with antipsychotics may benefit aged patients with psychiatric disorders and dementia with neuropsychiatric symptoms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data Availability

Data will be made available on request.

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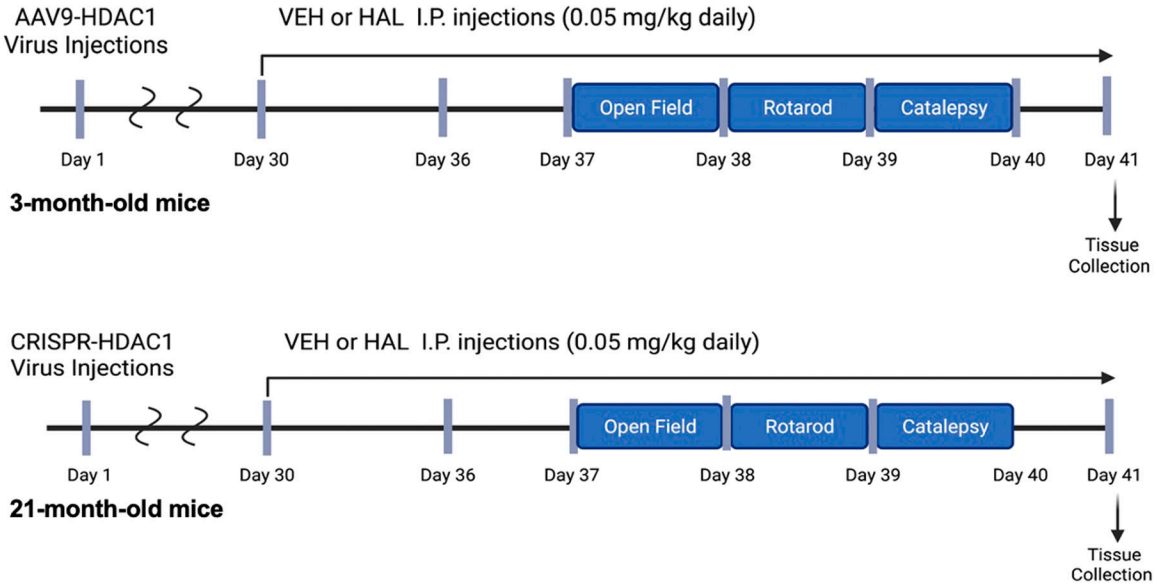

Fig. 1.

Schematic Illustration of Experimental Design. Young (3-month-old) mice and aged (21-month-old) mice were injected with either AAV9-HDAC1 or CRISPR-HDAC1 in the dorsal striatum. Mice were allowed one month to enable the virus to transfect and then were administered either haloperidol (0.05 mg/kg) or vehicle (VEH) daily for two weeks. During the last week of injections, mice were performed several behavioral tests to assess motor function 30 mins after haloperidol (HAL) administration. In addition, the striatum was collected for biochemical measurements on the last day of haloperidol administration.

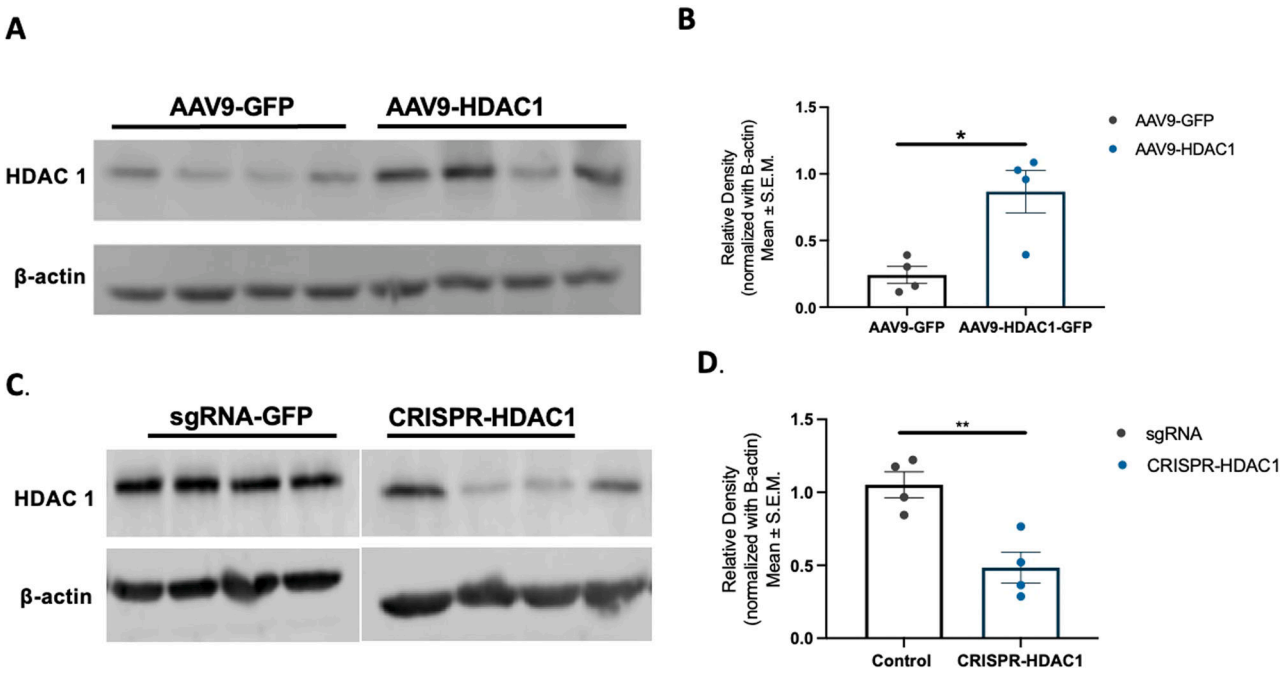

Fig. 2.

Overexpression or Knock Down of HDAC1 in the Striatum of Young or Aged Mice. A: Representative immunoblots displayed a significantly increased HDAC1 protein expression in the striatum of 3-month-aged mice compared to the control group after four weeks of AAV9-HDAC1 transfection. B: Immunoblot quantification of panel A. C: Representative immunoblots displayed a significantly decreased HDAC1 protein expression in the striatum of 21-month-aged mice compared to the control group after four weeks of CRISPR-HDAC1 transfection. D: Immunoblot quantification of panel C.

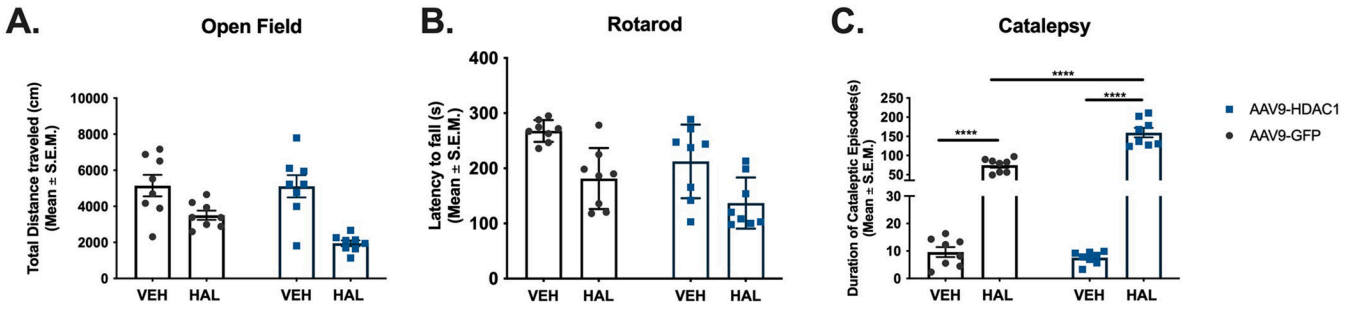

Fig. 3.

Impact of HDAC 1 Overexpression on Haloperidol-Induced Motor Side Effects in Young Mice. A: Open field test indicated a significant drug effect on general locomotive activity. B: Rotarod behavior test indicated a significant drug and virus effect, but not interaction on motor coordination. C: Catalepsy behavior test showed increased cataleptic behavior in AAV9-GFP+HAL, and AAV9-HDAC1+HAL treated mice compared to AA9-GFP+VEH, and AAV9-HDAC1 +VEH treated mice, however. AAV9-HDAC1+HAL treated mice showed a significant increase in cataleptic episodes. Four independent groups (AAV9-GFP+VEH, AAV9-GFP+HAL, AAV9-HDAC1+VEH, and AAV9-HDAC1 +HAL) of mice were used for this experiment. Data represent mean ± SEM (n = 8/group). *p < 0.05, * *p < 0.01, * ** *p < 0.0001.

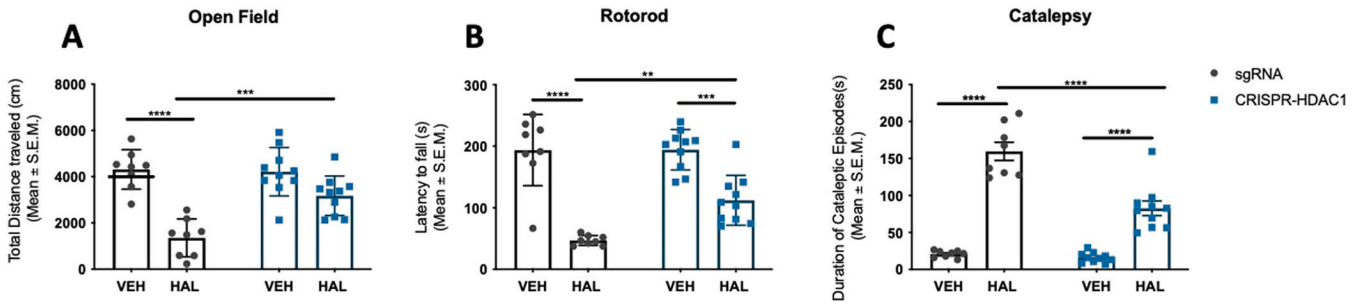

Fig. 4.

Impact of HDAC 1 Knock-Down on Haloperidol-Induced Motor Side Effects in Aged Mice. A: Open field test indicated locomotor deficits in scramble sgRNA+HAL mice but not in the CRISPR-HDAC1 +HAL mice. B: Rotarod behavior test also indicated motor coordination deficits in scramble sgRNA +HAL mice, but not CRISPR-HDAC1 +HAL mice. C: Catalepsy behavior test showed increased cataleptic behavior in scramble sgRNA+HAL and CRISPR-HDAC1 +HAL mice compared to scramble sgRNA+VEH and CRISPR-HDAC1 +VEH mice, CRISPR-HDAC1 +HAL mice showed a significant decrease in cataleptic episodes compared to scramble sgRNA+HAL mice. Four independent groups (sgRNA+VEH, sgRNA+HAL, CRISPR-HDAC1 +VEH, and CRISPR-HDAC1 +HAL) of mice were used for this experiment. Data represent mean ± SEM (n = 8–9/group). *$p < 0.05$, * *$p < 0.01$, * **$p < 0.001$ * ** *$p < 0.0001$