Histamine modulation of the basal ganglia circuitry in the development of pathological grooming

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Histamine (HA) modulates activity throughout the nervous system (1, 2). Histaminergic neurons are found in the tuberomammillary nuclei (TMN) of the posterior hypothalamus; they project broadly (1, 3, 4). The neurons fire rapidly during wakefulness and are nearly silent during sleep (5–7). TMN histaminergic neurons have recently been found to corelease GABA (8). HA is also produced in the gut (9) and by basophils and mast cells (10), including in the brain (11). Thus, HA from both neuronal and nonneuronal sources may modulate brain function.

HA dysregulation has been investigated in a range of neuropsychiatric pathophysiology (2, 12–14). It has recently emerged as a rare cause of Tourette syndrome (TS) and obsessive-compulsive disorder (OCD) (12, 13). A nonsense mutation in histidine decarboxylase (Hdc), which catalyzes the biosynthesis of HA from histidine, was associated with these conditions in a family with an exceptionally high incidence of both (15). Two other genetic studies suggest that disrupted HA signaling contributes to TS beyond this index family (16, 17). A recent analysis of Hdc knockout mice shows behavioral phenotypes and neurochemical alterations reminiscent of TS (18), establishing them as a plausible animal model of its pathophysiology.

Hdc knockout mice show elevated stereotypy after amphetamine challenge (18) or treatment with a HA H3 receptor agonist (19), and increased grooming after an acute stressor (20). Elevated grooming has garnered increasing interest in recent years as a potential behavioral model for compulsive behavior (21). It is seen in proposed mouse models of OCD (22–24), autism (25, 26), Rett syndrome (27), and trichotillomania (28). Repetitive stimulation of projections from the orbitofrontal cortex to the striatum, thought to be hyperactive in OCD, can produce elevated grooming (29).

TS and OCD are associated with abnormalities in the basal ganglia circuitry (30–32). HA receptors are particularly dense in the basal ganglia (1), and HDC protein is present at exceptionally high levels (33). Hdc knockout mice exhibit elevated markers of neural activity in the striatum, the primary input nucleus of the basal ganglia (18, 34). Ex vivo, HA modulates afferent glutamatergic inputs to the striatum and GABAergic inhibition within it (35). HA interacts with dopamine in the regulation of cell signaling in medium spiny neurons (MSNs) of the striatum in complex ways that are only beginning to come into focus (36–38).

How HA regulates processes of relevance to TS and OCD remains unclear. In carriers of the Hdc mutation (15) and in Hdc knockout mice (18), pathophysiological change could derive from HA disruption in the adult or during development; disease-relevant phenomenology could relate to dysregulation of the basal ganglia circuitry, the cortex, or other brain regions. We address these questions in the present study.

Materials and Methods

Detailed methods are provided in SI Materials and Methods. Hdc-cre knockout mice (https://jaxmice.jax.org/strain/021198) and wild-type sibling controls

Significance

Dysregulation of brain histamine (HA) is a rare cause of Tourette syndrome and related conditions, but the associated pathophysiological mechanisms remain obscure. We show that repetitive behavioral pathology in mice derives from deficiency of neurotransmitter HA (rather than HA from other sources), acting in the dorsal striatum. These data provide a functional dissection of acute HA modulation in the brain and elucidate the anatomical correlates of pathological dysregulation when neuronal HA is disrupted.

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were considered significant at antibodies and staining conditions are given in chemistry was performed as described previously (18, 34). Details of (Fig. 1 and Fig. S1 patches, suggestive of increased grooming; 0 of 6 littermate virus, and brain development is normal. unperturbed by this manipulation because of the targeting of the Hdc pleiotropic (41) into the posterior hypothalamus of adult, we infused a development. To isolate a deficit in neurotransmitter HA in the (18, 20, 34), lack both neuronal and peripheral HA throughout out mice (39, 40), which recapitulate characteristics of TS and OCD (see Figs. 2 A, B, and Movies S3 and S4). This effect was also seen during the animals’ dark cycle (Fig. S4 D and E). A similar effect was seen in a larger open-field environment: chemogenetic inhibition of

were used for all experiments. All experimental procedures and animal care were approved by and under the supervision of the Yale University Institutional Animal Care and Use Committee.

For targeted ablation of Hdc-expressing neurons, virus AAVS-Flex-taCas93-TEVp was infused into the TMN. For chemogenetic inactivation of Hdc-expressing neurons, virus AAV-hSyn-DIO-hM4D(Gi)-mCherry was similarly infused. For behavioral rescue with intrastral HA, mice were fitted with bilateral cannula guides targeting the central striatum at the time of viral infusion; HA or saline was infused at the time of behavioral testing (see Fig. 1).

For activity-dependent tagging and bidirectional chemogenetic control of HA-regulated neurons, we used an AAVE2-SARE-ER<sup>T2</sup>-CreERT<sup>2</sup>-PEST, AAV9-hSyn-DIO-HA-KORD-ires-mCherry, and AAVS-hSyn-DIO-hM3D(Gq)-mCherry (see Figs. 2 A and B and 3 A and B).

Microdialysis to measure intrastral HA was performed as previously described (18). Behavioral analysis was initiated 2–3 wk following viral infusion and followed previously published methods (18, 19). Immunohistochemistry was performed as described previously (18, 34). Details of antibodies and staining conditions are given in SI Materials and Methods.

Statistical analysis was performed using GraphPad Prism. All comparisons were considered significant at α = 0.05.

**Results**

**Elevated Grooming After Ablation or Chemogenetic Inhibition of Histaminergic Neurons in Developmentally Normal Mice.** Hdc knock-out mice (39, 40), which recapitulate characteristics of TS and OCD (18, 20, 34), lack both neuronal and peripheral HA throughout development. To isolate a deficit in neurotransmitter HA in the adult, we infused a Cre-inducible virus expressing a hybrid caspase (41) into the posterior hypothalamus of Hdc-Cre knockin mice (https://jaxmice.jax.org/strain/02198) (42), thereby specifically depleting Hdc-expressing neurons. Peripheral sources of HA are unperturbed by this manipulation because of the targeting of the virus, and brain development is normal.

This process reduced Hdc-expressing cells in the TMN ~50% (Fig. 1 and Fig. SLA). Nine of 10 ablated mice developed bald patches, suggestive of increased grooming; 0 of 6 littermate control mice developed similar skin lesions (Fig. SLB). Video analysis (Movies S1 and S2) confirmed increased grooming in ablated mice (Fig. 1). Grooming bouts were also increased. Elevated grooming was seen across the circadian cycle (Fig. SIC). One animal in the ablation group proved to have a normal number of Hdc-expressing neurons, normal grooming behavior, and no bald patches (Fig. 1, arrow, and Fig. SLA). Hdc-ablated animals showed modestly reduced anxiety and a trend toward increased startle, but no alterations in exploratory locomotion or prepulse inhibition (PPI) of startle (Figs. S2 and S3).

We replicated this effect using chemogenetic inhibition of Hdc-expressing neurons with the hM4Di DREADD (designer receptors exclusively activated by designer drugs) receptor (43, 44). Cre-dependent hM4Di-expressing virus was delivered into the TMN in Hdc-Cre transgenic mice; activation of hM4Di by systemic administration of clozapine N-oxide (CNO) reduced striatal HA, measured by in vivo microdialysis (Fig. 2A), confirming the inhibition of histaminergic neurons. This was associated with markedly increased grooming (Fig. 2B, Fig. S4 A and B, and Movies S3 and S4). This effect was also seen during the animals’ dark cycle (Fig. S4 D and E). A similar effect was seen in a larger open-field environment: chemogenetic inhibition of

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![Image](https://example.com/image.png)

**Fig. 1.** Increased grooming after histaminergic neuron ablation. Hdc<sup>−</sup> cells were reduced in Hdc-Cre mice (t(13) = 5.64, P < 0.0001). Hdc-Cre mice exhibited elevated grooming, scored as total seconds across three 20-min blocks spaced across 24 h (Fig. S1) (t(13) = 3.91, P = 0.0018). Across all animals, grooming and total number of Hdc<sup>−</sup> cells were negatively correlated (r = −0.63, P < 0.01). Blue arrow indicates the one animal in the Hdc-Cre group in which ablation was minimal (Fig. S1B, Right).

![Image](https://example.com/image.png)

**Fig. 2.** Increased grooming and dStr and mPFC activity in after chemogenetic inhibition of TMN HA neurons. (A) Inactivation of TMN Hdc-expressing neurons using the hM4Di DREADD led to a significant reduction in striatal HA, measured by microdialysis, over the 5 h following CNO injection (n = 6; one-way repeated-measures ANOVA: F(5, 25) = 10.45, P < 0.0001). Significance indicators reflect post hoc comparisons to baseline, using Dunnett’s test. (B) CNO injection produced elevated grooming across 6 h [ANOVA: drug, F(1, 26) = 14.06, P < 0.001; cre, F(1, 26) = 6.05, P = 0.021; interaction, F(1, 26) = 6.51, P = 0.017]. (C) In a larger open-field environment, chemogenetic inactivation of Hdc<sup>−</sup> cells produced elevated stereotyped beam-breaks [t(13) = 2.5, P = 0.026] but no significant change in ambulatory beam-breaks [t(13) = 0.97, P = 0.35]. (D) Striatal zif268 was up-regulated in the dStr after CNO treatment. Mann-Whitney U = 12; P = 0.0375; n = 8 per group. (E) Striatal c-fos was also up-regulated in the dStr after CNO treatment: t(11) = 4.79, P = 0.0006; n = 7 saline, 6 CNO. (F) Because c-fos gave the cleaner result in the dStr analysis we selected its count for in the mPFC. c-fos<sup>−</sup> cells were elevated in prelimbic (PL) and infralimbic cortices (IL) but not in cingulate after TMN HA cell inactivation. A 2 × 3 repeated-measures ANOVA: main effect of CNO: F(1, 11) = 38, P < 0.0001; main effect of PFC subregion, F(2, 22) = 26.3, P < 0.0001; interaction, F(2, 22) = 25.6, P < 0.0001; n = 7 saline, 6 CNO. Post hoc comparisons indicate difference from the same subregion in saline-treated animals, using Sidak’s test. *P < 0.05; **P < 0.01; ***P < 0.001; ****P ≤ 0.0001.
Hdc-expressing neurons in an open field increased stereotypic beam-breaks, but not ambulatory beam-breaks (Fig. 2C).

Elevated Striatal and Cortical Activity After TMN Inhibition. Grooming is associated with neuronal activity in the striatum (21, 45, 46). We examined the immediate early genes (IEGs) c-fos and egfl1a1f268 in the dorsal striatum (dStr) (Fig. S5A) in TMN hM4D-expressing mice injected with either saline or CNO 1 h before being killed. Both IEGs were up-regulated in the striatum (Fig. 2D and E). Because c-fos gave the cleaner signal, we also analyzed it in the medial prefrontal cortex (mPFC); c-fos+ cells were also increased after TMN HC cell inactivation in the infralimbic and prelimbic cortex, but not in the cingulate cortex (Fig. 2F and Fig. S5B).

Activity of HA-Regulated Cells in the dStr Is Sufficient to Elevate Grooming. We hypothesized that this dStr activity is sufficient to produce elevated grooming; we tested this hypothesis using a dual chemogenetic approach (47). DREADDs have previously been documented to bidirectionally regulate MSNs (43).

Targeting of DREADD expression to active cells was achieved using a recently described activity-driven, 4-OH-tamoxifen (4-OH-TMX)–gated Cre recombinase that is far more sensitive and less leaky than previous systems that have been used for this purpose (48). The hybrid E-SARE promoter serves a function similar to that of the Fos promoter in previous studies (49–52) but has a substantially better signal-to-noise ratio and dramatically reduced leakiness (48, 53). The ER12–CreER12–PEST fusion protein has a reduced half-life, relative to unmodified Cre recombinase, because of addition of the PEST sequence (48); this improves the temporal specificity of the system. Temporal precision is further enhanced through use of 4-OH-TMX, which has a shorter half-life than tamoxifen (54, 55). The time-frame over which 4-OH-TMX–induced recombination is optimal (5–9 h) matches well with the time-frame over which we see elevated grooming after TMN inactivation (26 h). When 4-OH-TMX injection was not paired with TMN inactivation, only sparse striatal cells were tagged with the hM3 DREADD (Fig. S6A), and CNO injection produced no significant behavioral effects (19) (key data reproduced in Fig. S6 B and C).

The kappa-opioid receptor (KORD) DREADD receptor was expressed in histaminergic neurons of the TMN in Hdc-cre transgenic mice through infusion of virus AAV9-hSyn-DIO-KORD-IRE5-mCitrine. Virus AAV2-E-SARE-ER12-CreER12–PEST and virus AAV5-hSyn-DIO-hM4D(Gi)-mCherry, which when activated by Cre-mediated recombination express the activating Gq-coupled hM3Dq DREADD receptor, were coinjected into dStr (Fig. S4). Two weeks following surgery, 4-OH-TMX was paired with Sandell B (SalB) to target DREADD expression in the dStr to activated cells (Fig. 3B and Fig. S7A). Grooming was assayed following saline, SalB, and CNO injection, at ~1-wk intervals (Fig. 3B). SalB and CNO produced similarly elevated grooming in all mice (Fig. 3C), demonstrating that activity of tagged dStr neurons is sufficient for the production of increased grooming.

Similar DREADD expression was seen when the E-SARE-ER12-CreER12 and DREADD viruses were infused into the mPFC (Fig. S7B). In these mice, SalB again led to increased grooming; but CNO, which activates tagged mPFC neurons, did not (Fig. 3D).

dStr Neural Activity Is Necessary for Elevated Grooming after TMN Inactivation. We used a variant of this strategy to probe the necessity of striatal activity for the production of elevated grooming after TMN inactivation (Fig. 4A and B). hM4D was expressed in histaminergic neurons in Hdc-cre transgenic mice by infusion of the virus AAV5-hSyn-DIO-hM4D(Gi)-mCherry into the posterior hypothalamus. Phasically activated dStr cells were tagged with the inhibitory KORD DREADD through the coinfusion of viruses AAV2-E-SARE-ER12-CreER12–PEST and AAV5-hSyn-DIO-hM4D (Gi)-mCherry, followed 2 wk later by coinjection of CNO and 4-OH-TMX (Fig. 4B).

Fig. 3. Sufficiency of striatal activity for the production of grooming. (A) Virus hSyn-DIO-hKORD-IRE5-mCitrine-WPRE-PolyA-R-ITR was infused bilaterally into the posterior hypothalamus of Hdc-cre transgenic mice, to achieve specific expression of the inhibitory KORD DREADD in histaminergic neurons. During the same surgery, two viruses were infused bilaterally into dStr or mPFC in separate groups of mice: AAV2-E-SARE-ER12-CreER12–PEST, for activity-dependent expression of tamoxifen-gated Cre recombinase (48), and AAV5-hSyn-DIO-hM3D(Gq)-mCherry for recombination-dependent expression of the Gq-coupled activating hM3 DREADD. (B) Experimental sequence for testing of sufficiency of dStr neuronal activity for grooming and locomotor behaviors. (C) In mice with dStr targeting of hM3D, both SalB (which inactivates TMN neurons) and CNO (which activates tagged dStr neurons) produced elevated grooming. Repeated-measures ANOVA: main effect of treatment, F(1, 23, 7.40) = 24.5, P = 0.0011. (D) In mice with mPFC targeting of hM3D, SalB induced elevated grooming, reconfirming the effect of TMN cell silencing, but CNO did not. Repeated-measures ANOVA (with Geisser–Greenhouse correction): main effect of treatment, F(2, 12) = 64.1, P < 0.0001. The differential effect of drug was confirmed in an omnibus analysis across both groups of animals: 2 × 3 repeated-measures ANOVA: main effect of drug, F(2, 24) = 64.2, P < 0.0001; main effect of anatomical target, F(1, 12) = 12.1, P = 0.0046; interaction, F(2, 24) = 11.3, P = 0.0004. The effect of CNO differed between dStr and mPFC experiments (C vs. D): t(12) = 5.3, P = 0.0002. n = 7 animals per group. Tukey post hoc (on one-way ANOVA analyses), compared with same-group saline: **P < 0.01; ***P < 0.001; n.s., not significant.
Inactivation of histaminergic neurons in the TMN again produced a dramatic elevation in grooming. When SalB was co-injected, inhibiting tagged dStr cells, grooming was significantly attenuated (although it remained elevated relative to saline) (Fig. 4C). In contrast, when cortical neurons were tagged with the KORD DREADD there was no significant attenuation of grooming when CNO and SalB were co-injected (Fig. 4D).

Striatal HA Reverses Elevated Grooming After TMN Inactivation. To test whether the striatum-dependent increase in grooming after TMN inactivation depends on HA (rather than, for example, coreleased GABA) (8), we repeated the chemogenetic inhibition of Hdc-expressing neurons in a separate cohort of mice, paired with bilateral infusion of either saline or HA (10 μg each side in 0.5 μL saline). Intracerebroventricular (ICV) infusion of 20 μg HA reduces locomotion (18), but we observed no such effect after intrastratial HA (Fig. S8), confirming that infused HA remained localized. Striatal HA infusion blocked the increased grooming seen after CNO (Fig. 4E).

Activation of mPFC Cells Regulated by TMN Inhibition Produces Locomotion. Finally, we tested whether chemogenetic activation of neurons activated after TMN inhibition (Fig. 3D) would regulate activity in a larger open field. Chemogenetic activation of tagged cortical cells led to markedly increased locomotion (Movies S5 and S6), whereas activation of tagged striatal cells led to nominally decreased locomotion (Fig. 5). This finding confirms the functionality of hM3D DREADD in tagged mPFC neurons.

Discussion
We provide in vivo evidence for a functional role for HA modulation of the cortico-striatal circuitry. Histaminergic modulation of the basal ganglia is of substantial importance in health and disease (2, 13, 18, 33, 36, 37, 56). This is highlighted by the recent
association of abnormal histaminergic neurotransmission with TS and OCD (12, 15–17), related and often comorbid conditions that are characterized by repetitive behaviors and by dysregulation of the cortico-basal ganglia circuitry (30–32).

Ablation or chemogenetic inhibition of Hdc-expressing cells in the TMN produces elevated grooming (Figs. 1 and 2). This is a more dramatic effect than in the chronically HA-deficient Hdc knockout mouse, in which repetitive behavioral pathology is seen only after psychostimulant challenge or acute stress (18, 20). The development of aberrant grooming after both cell ablation and chemogenetic inhibition, with either hM4D or KORD, supports a critical role for modulation of repetitive behavior by the TMN.

Our chemogenetic strategy allowed us to dissect the differential behavioral effects of TMN silencing in different brain regions. We use an activity-regulated, ligand-gated Cre recombinase (48) to tag cells in the dStr or mPFC, expressing DREADD receptors in those cells whose activity is increased after TMN inhibition (Fig. S7). This process allows us to inhibit TMN neurons with one DREADD ligand and then, independently, to activate or inhibit tagged dStr or mPFC neurons with another ligand.

We find a dissociation of the behavioral consequences dStr and mPFC activity after TMN inhibition. Chemogenetic activation of dStr neurons tagged after TMN inactivation leads to markedly elevated grooming (Fig. 3C). This effect is not seen when the hM3Dq DREADD is targeted to the mPFC (Fig. 3D).

Importantly, these data do not define a unique population of dStr cells involved in this effect, nor do our conclusions require that such a unique population exists: the precise subset of dStr cells activated by TMN inhibition may vary depending on other, uncontrolled variables, such as environmental context or the animal’s behavioral or motivation state. That said, chemogenetic inhibition of tagged dStr neurons attenuates the elevated grooming seen after concurrent TMN inactivation (Fig. 3C), demonstrating the necessity of the tagged dStr cells for full expression of the aberrant grooming phenotype. This effect was not observed when tagged mPFC cells were silenced.

Chemogenetic activation of mPFC cells has no effect on grooming, but instead induces hyperlocomotion (Fig. 5 and Movies S5 and S6). No similar effect is seen when tagged dStr cells are activated; indeed, the trend is toward lower exploratory grooming in the dStr group, consistent with a competition between hyperlocomotion and grooming behavior.

GABAergic cotransmission has recently been documented in TMN histaminergic neurons. Elimination of GABA synthesis in TMN neurons was found to disinhibit cortical neurons and to produce increased arousal and hyperactivity (8). Indeed, this mechanism may well explain the elevated cortical activity seen after TMN inhibition (Fig. 2F) and the elevated locomotor activity seen when these cells are later chemogenetically activated (Fig. 5).

However, our data suggest that this is not true in the striatum, and that elevated grooming after TMN inhibition is a result of disruption of HA transmission, not GABAA cotransmission: elevated grooming is reversed by bilateral infusion of HA into the striatum (Fig. 4E). A similar conclusion is supported by studies in the Hdc knockout mouse, in which stereotypic behavior is produced by activation of histaminergic signaling in the dorsal striatum (19).

TMN afferents to the striatum are sparse and varicose and produce few synapses (1), and are thus thought to produce widespread changes in tonic neurotransmitter levels rather than precise modulation of specific neurons. Striatal GABA derives from multiple afferents, interneurons, and MSNs themselves. Hence, it is unlikely that removal of a single source of tonic GABA could lead to such dramatic dysregulation. Indeed, the removal of TMN GABA has been shown to produce only subtle effects on striatal electrophysiology (8).

This double dissociation between the dStr and mPFC effects of TMN silencing provides new insight into the separable behavioral effects of TMN modulation of discrete brain circuits. It remains unclear whether the same TMN cells project to both the mPFC and dStr but produce disparate effects, or whether distinct TMN populations project to these two targets. Classic neuroanatomical studies have described three histaminergic projections from the TMN, two ascending and one descending (1). Some indirect evidence has been interpreted as implying that the histaminergic cells that innervate the striatum are a distinct population from those that innervate other forebrain structures (57, 58), but this remains to be clarified.

Our data identify the dStr as a mediator of elevated grooming after TMN inhibition. The specific molecular and cellular mechanisms that underlie this effect remain to be determined. Glutamatergic afferents to the striatum are negatively regulated by the H3R HA receptor (35, 59, 60); reduced HA acting at these receptors may disinhibit these excitatory afferents. In addition, postsynaptic H3R has recently been shown to modulate activity-dependent neuronal signaling in MSNs in complicated ways (36–38); we have found H3R activation to antagonize some effects of dopamine on neuronal signaling in the striatum (38). Reduced HA levels may thus potentiate effects of dopamine on this circuitry. The Ga3-coupled H2R receptor, on the other hand, activates MSNs (35); loss of HA tone at this receptor would be expected to reduce MSN activity and is thus less likely to mediate the effects we see here.

Mutation of the Hdc gene is a rare cause of TS, with comorbid OCD in many cases (15). We have previously demonstrated that Hdc knockout mice have phenomenological and neurochemical abnormalities that parallel those seen in TS patients (18). The current results add mechanistic clarity to this association, establishing that that either acute or chronic disruption of dStr modulation by neurotransmitter HA derived from the posterior hypothalamus is sufficient to produce repetitive behavioral pathology, which may model core symptomatology of TS and OCD (21, 61). This argues that the effects seen after Hdc knockout are attributable, at least in large part, to a deficiency of neurotransmitter HA in the adult brain, rather than to developmental effects or to the lack peripheral HA.

Elevated grooming has been described after a number of genetic and circuit-level manipulations, and in models that seek to capture the pathophysiology of a range of neuropsychiatric conditions (21, 61). For example, genetically modified mice that exhibit an elevated grooming phenotype have been described as models of OCD (22–24), Rett syndrome (27), autism (25, 26), and other conditions, as well as in our own work on tics and TS (19, 20). This finding emphasizes that elevated grooming is not isomorphic to any specific neuropsychiatric symptomatology. In this it resembles phenotypes, such as elevated locomotion, which can arise in a variety of physiological and pathophysiological contexts (and yet be very informative when analyzed carefully). It is increasingly clear that dysregulated grooming can derive from abnormalities in the cortico-basal ganglia circuitry (21, 45, 46); our results provide further support for this conclusion. Striatal overactivation has previously been associated with elevated grooming and alterations in grooming syntax (46, 62), and opotogenetic stimulation of afferents to the striatum can bidirectionally modulate grooming (29, 63). Our findings are therefore best interpreted not as implying that histaminergic dysregulation is specific to the pathophysiology of TS and OCD or that the elevated grooming documented here is perfectly isomorphic to tics, but rather as demonstrating more generally that perturbation of histaminergic modulation of the basal ganglia can lead to repetitive behavioral pathology, of potential relevance to a range of neuropsychiatric disorders.

These results identify a potential causal locus of HA’s contribution to pathological grooming and neuropsychiatric disorders characterized by repetitive behavioral pathology, such as TS and OCD. Further clarification of these mechanisms, in humans, holds promise for the further elucidation of pathophysiology and for the development of novel therapeutic strategies.
The generation of Hdc-cre mice has been previously described (42); Cre recombinase is expressed exclusively in histaminergic cells of the posterior hypothalamus in adults, with no recombination detected in the striatum, cortex, or other brain regions. (Developmental expression is seen in other brain regions, including the thalamus, but this is not seen in the adult and thus does not affect our experiments.) Hdc-cre+ and Hdc-cre− mice were produced by crossing hemizygous Hdc-cre− mice and in Fig. S5. These cells were and 4 siblings 1.5 mm; DV: 0.5 mm; AP: ±1.5 mm; L: ±1.5 mm; DV: −2.2 mm; 0.2 μL, together with either virus AAV5-hSyn-DIO-hM3D(Gq)-mCherry (Fig. 3 and Fig. S6) or virus AAV9-hSyn-DIO-HA-KORD-ires-mCitrine (Fig. 4). After 2 wk for recovery and viral expression, SunB (Fig. 3 and Fig. S6) or CNO (Fig. 4) was administered together with 4-OH-TMX (all intraperitoneally); this led to recombination of hM3D (Fig. 3 and Fig. S6) or KORD virus (Fig. 4) in a subset of cells in the dStr or mPFC, as shown in Fig. 3 C and E and in Fig. S5. These cells were subsequently regulated by intraperitoneal injections of CNO and SunB, as illustrated schematically in Figs. 3B and 4B.

Microdialysis. Microdialysis (Fig. 2A) was performed as previously described (18). Virus AAV5-hSyn-DIO-hM4D(Gi)-mCherry was infused into the posterior hypothalamus of Hdc-cre transgenic mice, as described above. During the same surgery, guide cannulae (CMA Microdialysis) were implanted unilaterally, targeting the dStr [AP: +0.5 mm; mediolateral (ML): ±1.5 mm; DV: −2.4 mm] (68). Cannulae were fixed to the skull using dental cement. Following 2 wk for recovery and viral expression, a microdialysis probe (2 mm CMA-7, 6-kDa cut-off; CMA Microdialysis) was inserted through the guide cannula to a depth of −4 mm relative to bregma (i.e., 2 mm below the tip of the guide cannula). Mice were left in the home cage for 1 h to habituate. Following this period, artificial cerebrospinal fluid (Harvard Apparatus) was continuously infused through the microdialysis cannula at a rate of 0.5 μL/min using a programmable infusion pump (CMA Microdialysis) to establish a baseline. CNO was then administered intraperitoneally, with continuous artificial cerebrospinal fluid perfusion; microdialysate for quantification of post-CNO HA levels was collected beginning 20 min after CNO, for 1 h. Dialysate was collected in perchloric acid and EDTA on ice. Samples were stored at −80 for later quantification of HA levels by mass spectrometry, following an established protocol and quality assurance measures (https://www.brains-online.com).

Behavioral Procedures. Behavioral testing started 2 wk after surgery to allow recovery and robust viral expression. Locomotor activity testing (Fig. 2C and Fig. 5B). Animals were placed into the behavioral room 20 min before testing, to briefly habituate. Next, mice were placed into the locomotor activity cage (18.5-inches length by 14.5-inches width by 8 inches height; Omnitech Electronics). For mice with TMN ablation (Fig. 1), activity was recorded for 1 h and then mice were returned to their

Supporting Information

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SI Materials and Methods

Mice. The generation of Hdc-cre mice has been previously described (42); Cre recombinase is expressed exclusively in histaminergic cells of the posterior hypothalamus in adults, with no recombination detected in the striatum, cortex, or other brain regions. (Developmental expression is seen in other brain regions, including the thalamus, but this is not seen in the adult and thus does not affect our experiments.) Hdc-cre+ and Hdc-cre− mice were produced by crossing hemizygous Hdc-cre− mice and in Fig. S5. These cells were and 4 siblings 1.5 mm; DV: 0.5 mm; AP: ±1.5 mm; L: ±1.5 mm; DV: −2.2 mm; 0.2 μL, together with either virus AAV5-hSyn-DIO-hM3D(Gq)-mCherry (Fig. 3 and Fig. S6) or virus AAV9-hSyn-DIO-HA-KORD-ires-mCitrine (Fig. 4). After 2 wk for recovery and viral expression, SunB (Fig. 3 and Fig. S6) or CNO (Fig. 4) was administered together with 4-OH-TMX (all intraperitoneally); this led to recombination of hM3D (Fig. 3 and Fig. S6) or KORD virus (Fig. 4) in a subset of cells in the dStr or mPFC, as shown in Fig. 3 C and E and in Fig. S5. These cells were subsequently regulated by intraperitoneal injections of CNO and SunB, as illustrated schematically in Figs. 3B and 4B.

Microdialysis. Microdialysis (Fig. 2A) was performed as previously described (18). Virus AAV5-hSyn-DIO-hM4D(Gi)-mCherry was infused into the posterior hypothalamus of Hdc-cre transgenic mice, as described above. During the same surgery, guide cannulae (CMA Microdialysis) were implanted unilaterally, targeting the dStr [AP: +0.5 mm; mediolateral (ML): ±1.5 mm; DV: −2.4 mm] (68). Cannulae were fixed to the skull using dental cement. Following 2 wk for recovery and viral expression, a microdialysis probe (2 mm CMA-7, 6-kDa cut-off; CMA Microdialysis) was inserted through the guide cannula to a depth of −4 mm relative to bregma (i.e., 2 mm below the tip of the guide cannula). Mice were left in the home cage for 1 h to habituate. Following this period, artificial cerebrospinal fluid (Harvard Apparatus) was continuously infused through the microdialysis cannula at a rate of 0.5 μL/min using a programmable infusion pump (CMA Microdialysis) to establish a baseline. CNO was then administered intraperitoneally, with continuous artificial cerebrospinal fluid perfusion; microdialysate for quantification of post-CNO HA levels was collected beginning 20 min after CNO, for 1 h. Dialysate was collected in perchloric acid and EDTA on ice. Samples were stored at −80 for later quantification of HA levels by mass spectrometry, following an established protocol and quality assurance measures (https://www.brains-online.com).

Behavioral Procedures. Behavioral testing started 2 wk after surgery to allow recovery and robust viral expression. Locomotor activity testing (Fig. 2C and Fig. 5B). Animals were placed into the behavioral room 20 min before testing, to briefly habituate. Next, mice were placed into the locomotor activity cage (18.5-inches length by 14.5-inches width by 8 inches height; Omnitech Electronics). For mice with TMN ablation (Fig. 1), activity was recorded for 1 h and then mice were returned to their

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home cage. For mice with chemogenetic regulation of TMN, dStr, or mPFC (Figs. 2–5), mice were monitored for 30 min to establish a baseline and were then injected with saline, CNO, or SalB (intraperitoneally). Locomotor activity was recorded for 1 h. Locomotor activity was quantified with Fusion software (Omnitech Electronics).

**Quantification of grooming.** For the ablation experiment (Fig. 1), Hdc-cre mice and controls infused with AAV5-flex-taCas3-TEVp were individually housed a home cage for 24 h with continuous video monitoring (HomeCageScan, CleverSys). Although this system allows for automated quantification of grooming, we have found this automated scoring to be less sensitive than and to correlate imperfectly with manual scoring from video (67); we therefore scored grooming behaviors as described by Greer and Capacchi (22), with minor modifications. Grooming was scored from video by an observer blind to experimental condition for the first 20 min of each hour in three time blocks spanning the 24 h of data: 10:00 AM–2:00 PM, 4:00 PM–8:00 PM, and 9:00 PM–1:00 AM. An individual grooming bout was considered only when it contained all of the elements of a grooming sequence, and if it lasted more than 20 s without interruption of more than 15 s. Grooming sequences immediately following eating were not included.

For chemogenetic experiments, mice were briefly (20 min) habituated to the behavioral room, and then injected intraperitoneally with CNO or SalB or with saline. Twenty minutes after this injection, mice were placed in a home cage in the CleverSys apparatus for 6 h. Movies were recorded during the day phase (10:00 AM–2:00 PM) or night phase (9:00 PM–1:00 AM). For mice with chemogenetic regulation of TMN, dStr, or mPFC (Figs. 2–5), mice were monitored for 30 min to establish a baseline and were then injected with saline, CNO, or SalB (intraperitoneally). Locomotor activity was recorded for 1 h. Locomotor activity was quantified with Fusion software (Omnitech Electronics).

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Fig. S1. Related to Fig. 1. Grooming after TMN neuron ablation in Hdc-cre transgenic mice. (A) HDC-immunopositive cells in the TMN were reduced in number in Cre+ mice 15 d after injection of AAV5-Flex-taCasp3-TEVp. (Magnification, 100×.) (B) Nine of 10 Hdc-cre mice in which HA neurons of the TMN were ablated developed dorsal bald patches 2 wk after virus injection, compared with 0 of 6 controls \((\chi^2 = 12.34, P < 0.0005)\). The one animal in the Hdc-cre group in which ablation was minimal (indicated by an arrow in Fig. 1) showed normal grooming and was the only animal in this group not to develop bald patches. (C) Grooming was scored in three 4-h blocks over the course of the 24-h light/dark cycle, to test whether circadian alterations in HA modulate the effect of neuronal ablation on grooming. There was a main effect of genotype \([\text{ANOVA: } F(1, 14) = 15.21, P = 0.0016]\) and a trend-level effect of time \([F(2, 28) = 2.96, P = 0.069]\) but no interaction \([F(2, 28) = 0.18, \text{n.s.}]\). The total grooming values given in Fig. 4 are the sum of the three time blocks here. Note that the number is larger here (10 rather than 9 animals in the ablated group) because HDC immunostaining failed for one animal, which is therefore excluded from the analysis in Fig. 1 but included here. (D) Total grooming bouts were significantly elevated in ablated mice relative to controls \([t(14) = 2.47, P = 0.027]\). (E) Analysis of grooming bouts across the three assayed time blocks showed a main effect of genotype \([F(1, 14) = 5.43, P = 0.035]\) and a marked effect of time \([F(2, 28) = 9.087, P < 0.001]\) but no interaction \([F(2, 28) = 0.94, \text{n.s.}]\). *\(P < 0.05\).
Fig. S2. Related to Fig. 1. Hdc genotype and Hdc-expressing TMN cell ablation do not affect locomotion. (A) Hdc-cre mice and wild-type controls (no viral infusion) were tested in the open field to ensure that there was no baseline difference in locomotion \(t(14) = 0.25, \text{n.s.}\). (B) After TMN Hdc cell ablation there was still no difference in locomotion, measured by beam breaks over 60 min [heteroskedastic \(t(10.3) = 1.05, \text{n.s.}\)].

Fig. S3. Related to Fig. 1. Anxiety, startle, and PPI of startle after Hdc-expressing TMN cell ablation. (A) In the elevated plus maze, a test of anxiety, mice with ablation of TMN Hdc cells spent more time in the open arm \(t(14) = 2.13, P = 0.051\) and less time in the closed arm [heteroskedastic \(t(11.6) = 2.24, P = 0.046\)] than controls, suggesting reduced anxiety. (B) Total entries into both open and closed arms did not differ between ablation mice and controls. (C) Mice with ablation of TMN Hdc neurons showed a trend toward elevated startle in the PPI apparatus [heteroskedastic \(t(8.96) = 2.18, P = 0.057\)]. In this they resemble Hdc knockout mice, which show elevated baseline startle (18). (D) In contrast, there was no change in PPI of the acoustic startle response after Hdc neuron ablation [repeated-measures ANOVA: main effect of genotype, \(F(1, 14) < 0.001, \text{n.s.}\); main effect of prepulse: \(F(2, 28) = 0.45, \text{n.s.}\); interaction, \(F(2, 28) = 0.43, \text{n.s.}\)]. In this these animals differ from Hdc knockouts (18). This finding suggests that, unlike repetitive movements, the PPI deficit observed in the knockouts derives from effects of longer-term HA deficiency or more profound HA deficiency, or from developmental effects of HDC loss that are not recapitulated in this model. \(^\dagger P < 0.1; \,* P < 0.05.\)
Fig. S4. Related to Fig. 2. Grooming after chemogenetic inhibition of Hdc-expressing TMN neurons. (A) Increased grooming after chemogenetic inhibition of TMN Hdc-expressing neurons; these are the same data as in Fig. S5B, collapsed to show mean and SEM. (B) The effect was marked in the first hour after CNO, although it did not reach statistical significance [two-tailed heteroskedastic t test: \(t(7.58) = 1.87, P = 0.10\)]. (C) In contrast, chemogenetic inhibition of TMN Hdc-expressing neurons did not produce an elevation in grooming bouts. [ANOVA: genotype, \(F(1, 26) = 1.17, \text{n.s.}\); CNO, \(F(1, 26) = 2.424, P = 0.13\); interaction, \(F(1, 26) = 0.73, \text{n.s.}\)]. (D) Elevated grooming was also seen after chemogenetic inhibition of TMN neurons during the animals' dark cycle, measured here across 6 h \(t(13) = 2.57, P = 0.024\). (E) Grooming in the first hour after CNO in the dark cycle \(t(13) = 2.35, P = 0.035\). †\(P < 0.1\); *\(P < 0.05\); **\(P < 0.005\).

Fig. S5. Related to Fig. 2. (A) Immunostaining for the immediate early genes zif268 (Upper) and c-fos (Lower) in the striatum. (Magnification, 40×.) Immunopositive cells were counted blind to experimental condition; a subset of positive cells are indicated by arrowheads, for illustrative purpose. (B) Anatomical definition of cingulate cortex, prelimbic cortex, and infralimbic cortex used for quantification of c-fos immunopositive cells in Fig. 2F; here, too, quantification was performed blind to experimental condition.
Fig. 56. Related to Fig. 3. Reduced DREADD tagging of dStr neurons and lack of behavioral effect of CNO when 4-OH-TMX is unpaired with TMN inactivation. 

(A) Viruses AAV2-E-SARE-ER<sup>12</sup>-CreER<sup>12</sup>-PEST and AAV5-hCyn-DIO-hM3D(Gq)-mCherry were infused into the dorsal striatum, as in Fig. 3; for this negative control experiment, no virus was infused into the TMN. 4-OH-TMX was administered 2 wk later, as in Fig. 3B, paired with systemic injection of saline but with no manipulation of the TMN. Only sparse scattered DREADD-expressing cells were detectable in dStr after this treatment (compare with Fig. S7A). (Magnification, 100×.) (B) There was no significant increase in stereotypic counts in the open field after CNO administration in these animals [n = 6; paired t test: t(5) = 0.83, P = 0.45]. (C) There was no change in ambulatory counts in the open field after CNO [paired t test: t(5) = 0.41, P = 0.70]. B is reproduced from data in figures 2A and 4A in ref. 19; WT<sub>saline</sub> condition; C reproduced from corresponding data from figures 2B and 4B in ref. 19.
Fig. S7. Related to Fig. 3. Tagging of activated neurons in dStr and mPFC following inhibition of Hdc-expressing TMN neurons. Coronal slices from the mice described in Fig. 3, with the hM3D DREADD expressed in activity-tagged cells in the dStr or mPFC, were immunostained for mCherry, which is coupled to the hM3D DREADD to ascertain expression and spread of recombinated DREADD virus. (A) hM3D-mCherry expression in dorsal striatum. (Upper) hM3D expression was seen in dStr cells of mice that received virus AAV5-HCyn-DIO-hM3D(Gq)-mCherry in the dStr. Low levels of hM3D expression when 4-OH-TMX was not paired with SalB are shown in Fig. S6A. (Lower) Viral spread in these animals. Pink indicates region of dense cellular staining; yellow indicates region of sparser staining that was more inconsistent across mice in the cohort. Images from four typical individual animals are shown. (B, Upper) When E-SARE-Cre and hM3D viruses were injected into mPFC, hM3D expression was again seen, documenting hM3D viral recombination. (Lower) Again, pink indicates area of dense and consistent expression, whereas yellow indicates region of sparser and more inconsistent staining. (Magnification, Upper micrographs in each panel, 100×; lower micrographs, 40×.)
Fig. S8. Related to Fig. 4. Intrastriatal HA does not affect locomotion in WT mice. We tested whether intrastriatal HA (10 μg on each side) would have general locomotor suppressive effects, as the same amount of HA does when injected ICV (18). HA injection had no effect on subsequent locomotor behavior; indeed, the trend was toward activation, although it did not reach statistical significance [t(9) = 1.68; P = 0.13].

Movie S1. Related to Fig. 1. Video of four mice (two ablated and two control) recorded in the CleverSys HomeCageScan apparatus illustrates elevated grooming in ablated mice, particularly in the animal in the lower left cage, as quantified in Fig. 1.
**Movie S2.** Related to Fig. 1. Video of four more mice (two ablated and two control), sped up 8× to permit efficient review of a longer time frame, illustrates increased grooming in both ablated (*Hdc-cre*⁺) mice relative to controls. This effect is quantified in Fig. 1 and Fig. S1.

**Movie S3.** Related to Fig. 3. Video of a mouse expressing hM3D in tagged dStr cells, as illustrated in Fig. 3, after saline injection. Note that this video has been sped up 4× and resolution has been downsampled for convenience.
Movie S4. Related to Fig. 3. Video of a mouse expressing hM3D in tagged dStr cells (the same animal as in Movie S3), after CNO injection. Grooming behavior is markedly increased, as quantified in Fig. 3B. Again, the video has been sped up 4x and resolution has been downsampled.

Movie S5. Related to Fig. 5. Video of a mouse expressing hM3D in tagged mPFC cells, as illustrated in Fig. 3, in an open field after saline injection.
Movie S6. Related to Fig. 5. The same mouse as in Movie S5, after CNO injection to activate tagged mPFC cells.