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

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Aberrant histaminergic function has been proposed as a cause of tic disorders. A rare mutation in the enzyme that produces histamine (HA), histidine decarboxylase (HDC), has been identified in patients with Tourette syndrome (TS). *Hdc* knockout mice exhibit repetitive behavioral pathology and neurochemical characteristics of TS, establishing them as a plausible model of tic pathophysiology. Where, when, and how HA deficiency produces these effects has remained unclear: whether the contribution of HA deficiency to pathogenesis is acute or developmental, and where in the brain the relevant consequences of HA deficiency occur. Here, we address these key pathophysiological questions, using anatomically and cellularly targeted manipulations in mice. We report that specific ablation or chemogenetic silencing of histaminergic neurons in the tuberomammillary nucleus (TMN) of the hypothalamus leads to markedly elevated grooming, a form of repetitive behavioral pathology, and to elevated markers of neuronal activity in both dorsal striatum and medial prefrontal cortex. Infusion of HA directly into the striatum reverses this behavioral pathology, confirming that acute HA deficiency mediates the effect. Bidirectional chemogenetic regulation reveals that dorsal striatum neurons activated after TMN silencing are both sufficient to produce repetitive behavioral pathology and necessary for the full expression of the effect. Chemogenetic activation of TMN-regulated medial prefrontal cortex neurons, in contrast, increases locomotion and not grooming. These data confirm the centrality of striatal regulation by neurotransmitter HA in the adult in the production of pathological grooming.

histamine | basal ganglia | grooming | striatum | Tourette syndrome

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* knockin 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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Fig. 1. Increased grooming after histaminergic neuron ablation. HDC⁺ 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⁺ 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).

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 AAV5-Flex-taCasp3-TEVp was infused into the TMN. For chemogenetic inactivation of *Hdc*-expressing neurons, virus AAV2-hSyn-DIO-hM4D(Gq)-mCherry was similarly infused. For behavioral rescue with intrastriatal 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 AAV2-E-SARE-ER^{T2}-CreER^{T2}-PEST, AAV9-hSyn-DIO-HA-KORD-IRES-mCitrine, and AAV5-hSyn-DIO-hM3D(Gq)-mCherry (see Figs. 2 A and B and 3 A and B).

Microdialysis to measure intrastriatal 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 $\alpha < 0.05$.

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/021198>) (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. S1A). Nine of 10 ablated mice developed bald patches, suggestive of increased grooming; 0 of 6 littermate control mice developed similar skin lesions (Fig. S1B). 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. S1C). 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. S1A). *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. S4A and B, and Movies S3 and S4). This effect was also seen during the animals' dark cycle (Fig. S4D and E). A similar effect was seen in a larger open-field environment: chemogenetic inhibition of

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 hM4i 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*⁺ cells produced elevated stereotypic 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 it for counting in the mPFC. *c-fos*⁺ 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. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P \leq 0.0001$.

Fig. 3. Sufficiency of striatal activity for the production of grooming. (A) Virus hSyn-DIO-hKORD-IRES-mCit-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-ER^{T2}-CreER^{T2}-PEST, for activity-dependent expression of tamoxifen-gated Cre recombinase (48), and AAV5-hCyn-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.

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) *egr1/zif268* and *c-fos* 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 HA 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 ER^{T2}-CreER^{T2}-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

(≥6 h). When 4-OH-TMX injection was not paired with TMN inactivation, only sparse striatal cells were tagged with the hM3D DREADD (Fig. S6A), and CNO injection produced no significant behavioral effects (19) (key data reproduced in Fig. S6B 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-HA-KORD-IRES-mCitrine. Virus AAV2-E-SARE-ER^{T2}-CreER^{T2}-PEST and virus AAV5-hSyn-DIO-hM3D(Gq)-mCherry, which when activated by Cre-mediated recombination express the activating Gq-coupled hM3Dq DREADD receptor, were coinjected into dStr (Fig. 3A). Two weeks following surgery, 4-OH-TMX was paired with Salvinorin B (SalB) to target DREADD expression in the dStr to activate 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-ER^{T2}-CreER^{T2} 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). hM4Di 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 coinjection of viruses AAV2-E-SARE-ER^{T2}-CreER^{T2}-PEST and AAV5-hSyn-DIO-hM4D(Gi)-mCherry, followed 2 wk later by coinjection of CNO and 4-OH-TMX (Fig. 4B).

Fig. 4. Necessity of striatal activity for grooming after TMN inactivation. (A) The Gi-coupled hM4D DREADD was expressed in histaminergic cells in the TMN, as in Fig. 3. The Gi-coupled KORD DREADD was expressed in activity-tagged cells in the dorsal striatum, as in Fig. 3. (B) Sequence of tests for this experiment. (C) Grooming was dramatically increased by CNO treatment, as before. This elevated grooming was significantly attenuated by coadministration of SalB, in mice in which the KORD DREADD was expressed in tagged dStr neurons. RM-ANOVA: $F(1.14, 5.69) = 25.5, P = 0.0024$. (D) When KORD was expressed in the mPFC, CNO again produced elevated grooming; coadministration of SalB had no significant effect, confirming the specificity of the requirement for striatal neuronal activity. Repeated-measures ANOVA: $F(1.14, 5.72) = 12.3, P = 0.013$. An omnibus ANOVA across both sets of animals confirmed the differential effect of drug in dStr vs. PFC mice: 2×3 repeated-measures ANOVA: main effect of drug, $F(2, 20) = 25.9, P < 0.0001$; main effect of anatomical target, $F(1, 10) = 4.6, P = 0.058$; interaction, $F(2, 20) = 5.3, P = 0.014$. The differential effect of SalB was further confirmed in a 2×2 ANOVA across only CNO and CNO + SalB conditions: main effect of drug, $F(1, 10) = 5.4, P = 0.042$; main effect of drug, $F(1, 10) = 1.8, P = 0.21$; interaction, $F(1, 100) = 5.02, P = 0.049$. $n = 6$ animals per group. Tukey's post hoc (on one-way ANOVA analyses): $*P < 0.05$, $***P < 0.001$, relative to saline; $#P < 0.05$ relative to CNO. (E) HA or saline was infused into the striatum bilaterally, simultaneously with inactivation of TMN *Hdc*⁺ neurons by CNO injection, thus depleting HA in the striatum but not in other TMN targets, such as the hippocampus and cortex. HA infusion reversed the elevated grooming seen after *Hdc* cell inactivation [ANOVA: genotype, $F(1, 28) = 5.51, P = 0.026$; HA vs. saline, $F(1, 28) = 4.48, P = 0.043$; interaction, $F(1, 28) = 4.31, P = 0.047$]. Among *Hdc*-cre mice, HA infusion reduced grooming (relative to saline infusion) in six of seven animals (Wilcoxon: $W = -24, P = 0.047$).

Inactivation of histaminergic neurons in the TMN again produced a dramatic elevation in grooming. When SalB was coadministered, 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 coadministered (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 μ g each side in 0.5 μ L saline). Intracerebroventricular (ICV) infusion of 20 μ g HA reduces locomotion (18), but we observed no such effect after intrastriatal 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

Fig. 5. Locomotor activation after activation of cortical neurons. (A) The inactivating KORD DREADD was expressed in histaminergic cells of the TMN, and cortical and striatal cells activated after TMN inactivation were tagged with the activating hM3D DREADD for subsequent chemogenetic activation (Fig. 3). (B) Activation of tagged cells in the mPFC led to increased locomotion, whereas activation of tagged cells in the dStr led to nominally decreased locomotion (although the latter effect did not reach statistical significance). Note that activation of tagged mPFC cells did not produce elevated grooming (compare with Fig. 3D, which is from the same animals). Repeated-measures ANOVA: main effect of drug, $F(1, 12) = 12.7, P = 0.004$; main effect of anatomical target, $F(1, 12) = 5.60, P = 0.036$; interaction, $F(1, 12) = 32.2, P = 0.0001$; $n = 7$ per group. Sidak's post hoc test (relative to same-group saline control): $****P \leq 0.0001$; n.s., post hoc comparison not significant.

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 locomotion in the dStr group, consistent with a competition between grooming and locomotor 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 GABA 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 neuro-anatomical 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 $G_{\alpha s}$ -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 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 optogenetic 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.

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