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Histidine decarboxylase knockout mice, a genetic model of Tourette syndrome, show repetitive grooming after induced fear

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Abstract

Tics, such as are seen in Tourette syndrome (TS), are common and can cause profound morbidity, but they are poorly understood. Tics are potentiated by psychostimulants, stress, and sleep deprivation. Mutations in the gene *histidine decarboxylase* (*Hdc*) have been implicated as a rare genetic cause of TS, and *Hdc* knockout mice have been validated as a genetic model that recapitulates phenomenological and pathophysiological aspects of the disorder. Tic-like stereotypies in this model have not been observed at baseline but emerge after acute challenge with the psychostimulant D-amphetamine. We tested the ability of an acute stressor to stimulate stereotypies in this model, using tone fear conditioning. *Hdc* knockout mice acquired conditioned fear normally, as manifest by freezing during the presentation of a tone 48 hours after it had been paired with a shock. During the 30 minutes following tone presentation they showed increased grooming. Heterozygotes exhibited normal freezing and intermediate grooming. These data validate a new paradigm for the examination of tic-like stereotypies in animals without pharmacological challenge and enhance the face validity of the *Hdc* knockout mouse as a pathophysiologically grounded model of tic disorders.

Keywords

Tourette syndrome; histidine decarboxylase; histamine; stress; stereotypical behavior; tics

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1. Introduction

Gilles de la Tourette syndrome is characterized by motor and phonic tics, which are defined as sudden, repetitive, nonrhythmic, involuntary or semi-involuntary movements (1, 2). Tourette syndrome (TS) represents the most severe end of a spectrum of tic disorders that, in aggregate, affect 5% of the population and produce substantial morbidity (3). Our understanding of the underpinnings of tics is very limited (4), as are the available treatments for severe disease (5, 6). Convergent data implicate abnormalities of the basal ganglia-thalamo-cortical circuitry in TS (4, 7). Progress has been hampered by a paucity of validated animal models in which to investigate pathophysiology (8-11).

TS is substantially genetic, though associated polymorphisms and causative mutations have proven elusive (12-14). A recent linkage study in a family with a high incidence of TS (in both children and adults) and an autosomal dominant inheritance pattern implicates a mutation in the histidine decarboxylase (*Hdc*) gene as a rare genetic cause (15). *Hdc* encodes the enzyme for the conversion of histidine into histamine (HA), both peripherally and in the central nervous system (16). Subsequent genetic analyses have implicated disruption of *Hdc* (17) or of histaminergic signaling more generally (18) in TS beyond the index family.

We have shown *Hdc* knockout mice to exhibit potentiated tic-like stereotypies, recapitulating core phenomenology of TS (19). These animals also parallel TS patients in that they have a deficit in prepulse inhibition (PPI; 19, 20) and dysregulated dopaminergic innervation of the basal ganglia (19, 21-23). Stereotypies are mitigated by pretreatment with the D2 antagonist haloperidol; D2 antagonists are the most efficacious pharmacotherapy for TS (5, 6). These findings validate the *Hdc* knockout mouse as a TS animal model with construct, face, and predictive validity (24). Nevertheless, the fact that stereotypies occur only after pharmacological challenge is a weakness of the model and complicates its use as a platform for the discovery of new therapies.

TS generally has a waxing and waning course, with periods of tic exacerbation alternating with periods of decreased tic severity (1, 2). Contextual variables, such as psychosocial stress, anxiety, emotional tension, and fatigue influence tic severity (25, 26). During periods of high psychosocial stress, tics tend to get worse. For example, in a large survey of 763 TS patients, both medical and social stressors commonly occurred within one year before tic onset. Specific stressors included fever, operations requiring general anesthesia, and stressful life events such as relocation or parental divorce or separation (27).

We examined whether acute stress could exacerbate tic-like stereotypies in the TS animal model, the *Hdc* knockout mouse. For this purpose we developed a novel paradigm for the assessment of stress-triggered stereotypies, using tone fear conditioning.

2. Materials and methods

2.1. Animals

All mouse experiments were approved by the Yale University Institutional Animal Care and Use Committee. Generation of the *Hdc* KO mice has been described elsewhere (28); the sequence from intron 5 to exon 9 was replaced with a neomycin phosphotransferase gene

cassette in the inverse orientation, leading to complete disruption of the endogenous gene. Knockout, heterozygote, and wild-type mice were bred in our vivarium from heterozygote breeders. Adult male mice, aged 6 – 8 months, were used in all experiments. Mice were housed in a temperature and climate-controlled facility on a 12-hour light/dark schedule.

2.2 Fear conditioning induced stress

Cued fear conditioning to a tone was induced using standard procedures (29). Fear conditioning experiments used aluminum chambers (30×20×25 cm) with grid floors controlled by MedPC software (Med Associates Inc., Georgia, VT) housed in a sound-attenuating outer chamber equipped with white noise generator, fan, and houselight. The fear conditioning session began with the activation of a house light. 2 minutes later a 30 s tone conditioned stimulus (CS) was activated, paired at the end with a 2 sec 0.75 mA foot shock unconditioned stimulus (US), with which it coterminated. A second, identical CS-US presentation followed after 90 s. Mice remained in the chamber for an additional 30 s after the second CS-US pairing, after which the house light was inactivated and mice were returned to their home cages.

Freezing and tone-induced grooming were assayed 48 hours later in a separate enclosure, a clear plastic box outside of the sound-attenuating chamber. Time spent grooming was scored from video by an observer blind to animal genotype for 30 min before and 30 min after presentation of the 30 sec white noise CS. After CS presentation, grooming was often fragmentary, consisting of repeated initiation of facial grooming that did not progress through the normal syntactic chain. This was occasionally superimposed on stereotypical movements such as neck-turning. Because normal grooming bouts do not always progress through the entire syntactic grooming chain, it was not possible to characterize individual grooming bouts in the KO mouse as normal or abnormal. All grooming was therefore scored in aggregate as a single measure, in total seconds, as has been done in other models (30, 31). Freezing was scored during the 30 sec CS presentation. Freezing was defined as the absence of all movement except respiration, assessed in one-second intervals.

2.3. Statistical analysis

Data were organized using Microsoft Excel and analyzed using SPSS (IBM). Because we anticipated that heterozygotes would be intermediate between knockouts and wild-type mice, we analyzed all data by Pearson correlation of the number of knockout alleles (0, 1, or 2 for WT, heterozygote, and knockout, respectively) against the total time spent grooming or the % time spent freezing. 2-tailed significance was set at $\alpha = 0.05$.

3. Results

At baseline (before the presentation of the CS), qualitatively normal grooming was observed. The time spent grooming was not correlated with the number of KO alleles ($r=0.081$; $p=0.727$; Figure 1A); this is consistent with our previous observation that the *Hdc* knockout mice do not have measurably increased stereotypy at baseline (19). During the 30 minutes after CS presentation, in contrast, grooming was increased in the KO animals. Grooming was correlated with the number of KO alleles ($r = 0.44$; $p = 0.046$; Figure 1B).

Comparison of before-CS and after-CS grooming revealed a trend towards increased grooming in wild-type mice that did not reach statistical significance (Figure 1C; paired t-test: $p = 0.2$) but statistically significant induction in the heterozygotes ($p = 0.014$) and knockouts ($p < 0.005$).

Freezing during CS presentation was not correlated with the number of KO alleles ($r=0.219$; $P=0.34$; Figure 1D). This indicates that foot shock fear learning is comparable between genotypes of *Hdc* mice.

4. Discussion

TS is characterized by motor and vocal tics, as well as by sensory and cognitive symptoms (1, 2). It affects 0.3% – 1.0% of children, with many improving as they age (3, 32); however, persistent disease in adulthood, such as is seen in carriers of a histidine decarboxylase mutation (19), can be a source of lifelong morbidity. *Hdc* deficiency has been identified as a rare genetic cause (15); we have validated *Hdc* knockout mice as an informative, pathophysiologically-grounded animal model of at least this genetic form of TS (19, 24).

Tics are often potentiated by acute or chronic stress (25-27), and they can be mitigated by therapies aimed at stress management or mitigation (33). We therefore tested the ability of acute stress, triggered by cued fear conditioning, to induce tic-like stereotypies in the *Hdc* knockout model. We find normal grooming and no detectable stereotypies at baseline, replicating our previous observations (19), but increased grooming and stereotypy during the 30 minutes following the stress induced by the presentation of a conditioned fear stimulus (Figure 1B). This increases the face validity of the *Hdc* mice as a model of TS, as it shows that they can exhibit enhanced tic-like behaviors in the absence of pharmacological challenge (19). This stress-induced stereotypy phenotype is more conducive to future pharmacological investigations than the previously documented amphetamine-induced stereotypy phenotype.

Importantly, the *Hdc* knockout mice exhibit normal freezing 48 hours after cued fear conditioning (Figure 1D). This implies that they have normal experience of the shock and fear memory and are therefore likely to experience a comparable level of stress after presentation of the tone. Consistent with their overall experience of stress being comparable to that of controls, we have previously shown them to exhibit normal anxiety-like behavior in the open field and elevated plus maze (19). Unimpaired fear memory has previously been documented in these animals – in fact, enhanced cued and contextual fear conditioning have been reported in these animals previously (34, 35). We do not see such an enhancement of fear learning in our animals. This may be because of differences in the fear training paradigm (e.g. we use two training trials, whereas previous studies have used five; ref. 27), because of other contextual differences (such as vivarium conditions), or because of differences in the genetic background of the mice. Notably, others have reported increased anxiety in *Hdc* knockout mice (36), which we have not replicated (19). This may suggest that our animals do not have the emotional alterations documented by others, due to differences in genetic background or contextual variables.

This novel paradigm for assaying stress-induced tic-like stereotypies has several advantages. The measurement of cued fear conditioning simultaneously with tic-like stereotypies provides a valuable internal control for the induction of stress. The measurement of grooming both before and after the induction of stress permits within-animal comparison to a baseline condition. The fact that stress is induced by a tone, without any physical manipulation of the animal, avoids confounds that might arise from other manipulations – for example, restraint stress is likely to induce grooming due to the physical manipulation.

HA is produced by neurons in the tuberomammillary nucleus of the posterior hypothalamus that project throughout the central nervous system (CNS) (16). Pharmacological studies show that enhancing central HA production modulates stereotypy produced by methamphetamine (37) or apomorphine (38). *Hdc* knockout mice exhibited potentiated tic-like stereotypies, recapitulating core phenomenology of TS; these were mitigated by the dopamine (DA) D2 receptor antagonist haloperidol, a proven pharmacotherapy, and by HA infusion into the brain (19). The mechanism whereby HA manipulations alter DA-regulated tics and stereotypies are unclear; one possibility is through modulation by HA of dopaminergic modulation of the basal ganglia, which have been implicated in stereotypies (39) and in TS (4, 7). HA modulates neuronal activity in the striatum, the input nucleus of the basal ganglia (40), and there is a particularly high level of HDC protein in the striatum (41), suggesting an important modulatory function for HA in this circuitry. Consistent with this view, we have documented altered immediate early gene expression in the striatum in *Hdc* knockout animals, both at baseline and after amphetamine challenge (19).

The mechanisms whereby acute stress can enhance grooming and stereotypy remain to be elucidated, as do the mechanisms whereby acute and chronic stress worsen symptoms in tic disorders (25-27). The major brain areas shown to be involved in contextual and cued fear conditioning include the amygdala, hippocampus, frontal cortex, and cingulate cortex. Abnormal brain activity has been documented in TS patients during tic generation in many of these regions, including the amygdala, and hippocampus; reduced activity has been seen in the anterior cingulate cortex (42). Such abnormalities may provide a substrate for interactions between stress and tic generation, though any such mechanistic relationship remains speculative.

Our finding of stress-induced stereotypy in the *Hdc* knockout mice thus supports their validity as a pathophysiologically informative model of TS (10, 19, 24). Further studies in these and related animals hold great promise for the elucidation of the pathophysiology of TS and the identification of potential new therapeutic targets.

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Highlights

- *Hdc* knockout mice have recently been validated as a genetic model of a rare genetic form of Tourette syndrome
- *Hdc* KO mice show increased grooming, compared to HET and WT mice, after fear stress
- *Hdc* KO mice show intact fear learning

Figure 1.

Acute stress-induced tic-like stereotypy in *Hdc* KO mice. **A.** Grooming and stereotypy (which was minimal) during 30 minutes prior to cued fear conditioning induced stress. **B.** Grooming and stereotypy during 30 minutes after cue presentation. There was a significant correlation between genotype (measured by # of KO alleles) and stereotypy. **C.** Induction of stereotypy by CS presentation varied across genotypes. **D.** Freezing during the 30-sec CS presentation, which did not differ among genotypes, confirms intact fear conditioning. N = 7 per group. * $P < 0.05$; *** $P < 0.005$.