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Research article

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

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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.

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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 manifested by freezing during the presentation of a tone 48 h after it had been paired with a shock. During the 30 min following tone presentation, knockout mice 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.

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.

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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-h 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 min later a 30 s tone conditioned stimulus (CS) was activated, paired at the end with a 2 s 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 h 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 s 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 also 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 s 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

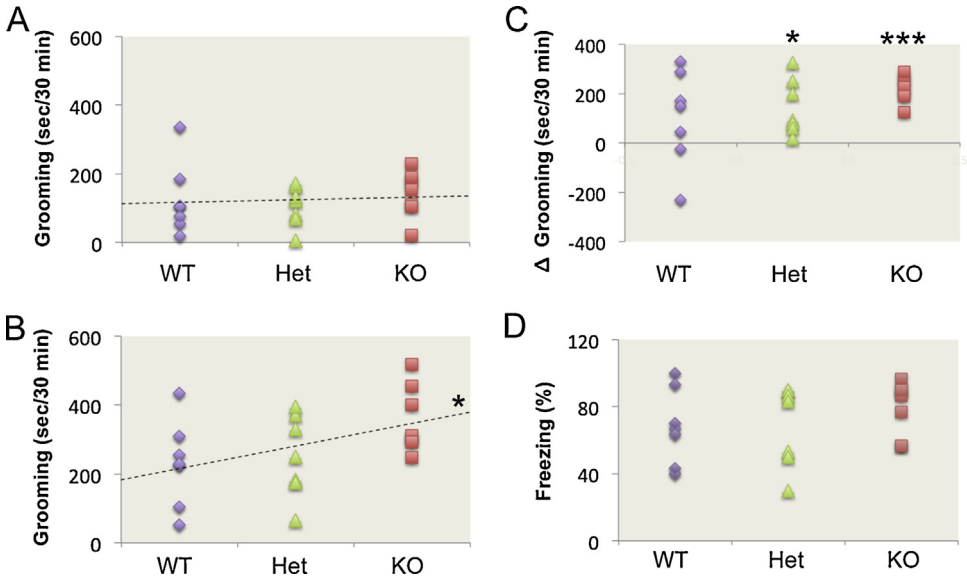

Fig. 1. Acute stress-induced tic-like stereotypy in *Hdc* KO mice. (A) Grooming and stereotypy (which was minimal) during 30 min prior to cued fear conditioning induced stress. (B) Grooming and stereotypy during 30 min after cue presentation. There was a significant correlation between genotype (measured by # of KO alleles) and grooming. (C) Induction of grooming by CS presentation varied across genotypes. (D) Freezing during the 30 s CS presentation, which did not differ among genotypes, confirms intact fear conditioning. $N = 7$ per group. $^{*}p < 0.05$; $^{***}p < 0.005$.