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Research Article

Tourette Syndrome: Do Reduced Histamine Levels Induce an Increase in Spontaneous Repetitive Behaviour?

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Abstract. Gilles de la Tourette syndrome (TS) is a disabling neuropsychiatric disorder characterised by persistent motor and vocal tics. Comorbidity of TS with other neuropsychiatric conditions such as obsessive compulsive disorder (OCD), attention deficit hyperactivity disorder (ADHD) and autism is frequent. TS has a significant genetic contribution and, in this regard, several susceptibility loci have been identified including the *histidine decarboxylase* (*HDC*) gene, which encodes an enzyme that is essential for histamine synthesis. Animal models of human disease are key to identify genetic and, importantly, pharmacological modifiers of phenotypes that mimic those present in the human condition. *HDC* is highly conserved throughout different species including the fruit fly *Drosophila melanogaster*. Aiming at uncovering TS-like phenotypes, in the present study we investigated repetitive grooming behaviour in flies that have reduced histamine levels as a result of a mutation in the *hdc*-encoding gene. We find that histamine deficiency in *Drosophila* is not associated with an increase in spontaneous repetitive grooming behaviour but rather a decrease. We speculate that the grooming behaviour in *Drosophila hdc* knockouts is not a translationally relevant TS phenotype. Future work should investigate whether stereotypy can be induced in the same mutants after pharmacological challenge or stress induction.

Keywords: Tourette Syndrome; *Drosophila*; grooming behaviour; histamine; histidine decarbyoxylase; model organism

Abbreviations

ADHD, attention-deficit hyperactivity disorder; *HDC*, *histidine decarboxylase*; OCD, obsessive-compulsive disorder; *SLITRK1*, *SLIT and TRK-like family member 1*; TS, Gilles de la Tourette syndrome

1 Introduction

Gilles de la Tourette syndrome (TS) is a disabling neuropsychiatric disorder where persistent motor and vocal tics are hallmark features. Comorbidity of TS with other neuropsychiatric conditions such as obsessive compulsive disorder (OCD), attention deficit hyperactivity disorder (ADHD) and autism is frequent, implying a common aetiology. There is as yet no cure for this disorder although pharmacological treatment and behavioural therapies can reduce symptoms. Twin studies have identified a significant genetic contribution to TS, where concordance rates over 50% were observed in monozygotic twins compared to rates below 10% for dizygotic twins (Hyde, Aaronson, Randolph, Rickler & Weinberger, 1992; Price, Kidd, Cohen, Pauls & Leckman, 1985). In addition, family studies show a greater rate of TS or chronic tics in first-degree relatives compared to rates in relatives of controls (Hebebrand et al., 1997; Kano, Ohta, Nagai, Pauls & Leckman, 2001; Pauls, Raymond, Stevenson & Leckman, 1991; Saccomani, Fabiana, Manuela & Giambattista, 2005). In this context, several association studies as well as linkage screens have identified and probed several TS susceptibility loci (Deng, Gao & Jankovic, 2012; O'Rourke, Scharf, Yu & Pauls, 2009; State, 2010, 2011).

Considerable excitement in the field was triggered by the discovery of two genes with a substantial role in TS including *L-histidine decarboxylase* (*HDC*), which encodes the rate limiting enzyme in histamine biosynthesis (Ercan-Sencicek et al., 2010), and the *SLIT and TRK-like family member 1* (*SLITRK1*), which encodes a transmembrane protein with strong homology to the axon guidance molecule, SLIT and the neurotrophin receptor, TRK (Abelson et al., 2005). Mouse knockouts

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of these two genes are available. Animal models are vital for modelling the human condition, thereby allowing the possibility of dissecting the function of disease-linked proteins, the uncovering of relevant disease pathways as well as the development and testing of novel therapeutic strategies. *Slitrk1*-knockout mice have a reduced body weight, slightly decreased viability, an elevated anxiety-like and depression-like behaviour. Although anxiety and depression are at times clinical features of TS, *Slitrk1*-null mice have no compulsive and/or tic-related behaviours. *Hdc*-knockout mice are viable but have reduced spontaneous locomotor activity in the dark as well as decreased exploratory activity in an illuminated open-field. Interestingly, under different testing conditions, *Hdc*-null mice display phenotypes that resemble features of TS including anxiety, potentiated tic-like stereotypic movements and increased grooming behaviour (Castellan Baldan et al., 2014; Cauchi & Tarnok, 2012; Xu, Li, Ohtsu & Pittenger, 2015).

In contrast to *SLITRK1*, *HDC* is highly conserved throughout different species including the fruit fly *Drosophila melanogaster* (Saenz-de-Miera & Ayala, 2004). *Drosophila* has a rich history with regards to its role in deciphering the genetic basis of behaviour and, in this regard, this model organism has been successful in modelling several neurological conditions including neuropsychiatric conditions such as schizophrenia, bipolar disorder and autism (Cauchi & van den Heuvel, 2006; Grice, Sleigh, Liu & Sattelle, 2011; Grice, Praveen, Matera & Liu, 2013; O'Kane, 2011; Sokolowski, 2001). *HDC* is essential for the production of histamine, which has diverse functions. In humans, histamine can act as a signalling molecule with important functions in gastrointestinal, immune, cardiovascular, respiratory and reproductive functions. In the nervous system, histamine acts as a transmitter with histaminergic neurons being involved in homeostatic brain functions and neuroendocrine control. Contribution to sensory and motor functions, cognition, attention, and learning as well as memory is also well-documented (extensively reviewed in Haas, Sergeeva & Selbach, 2008). In *Drosophila*, mutations in the *hdc* gene disrupt adult but not larval photoreceptor synaptic transmission and, hence, mutant flies are visually impaired in adulthood (Burg, Sarthy, Koliantz & Pak, 1993; Melzig et al., 1996, 1998). Furthermore, in view of various mechanosensory deficiencies displayed by *hdc* mutants, it was concluded that histamine is a major functional neurotransmitter for mechanosensory receptors. The *hdc* gene and other genes encoding for proteins involved in histamine signalling were recently identified in a screen for genes involved in sensing ambient temperature and in responding to its change (Hong et al., 2006).

Based on recent evidence implicating the *HDC* gene and histaminergic neural pathways in the aetiology of TS, we hypothesised that histamine deficiency induces TS-like behaviours including repetitive grooming. To this end, and aiming at developing a *Drosophila* model of TS, in the present study we investigated the spontaneous grooming behaviour of flies with a homozygous deficiency of the *hdc* gene.

2 Materials & Methods

2.1 Fly Stocks

Flies were cultured at 25 °C on standard molasses/maizemeal and agar medium in plastic vials under a 12 h/12 h light/dark cycle. The wild-type strain was *y w*. The previously-characterised hdc^{JK910}, hdc^{P218}, hdc^{P211} and hdc^{P217} are ethyl methanesulfonate (EMS) mutants of the *hdc* gene and were generous gifts from William Pak (Purdue University, West Lafayette, Indiana, USA) (Burg et al., 1993; Melzig et al., 1996, 1998).

2.2 Grooming Assay

Grooming assays of mutants and controls were conducted on the same day and during the daytime to minimise potential effects of circadian rhythm and climatic variations. Single male flies were transferred into a well-illuminated 1 cm^3 observation chamber, allowed to acclimatise in the new environment for 5 minutes, and were then video-recorded by a Sony DCR-SX53E video camera for 5 minutes. The captured video was analysed by one observer (B.A.). Video annotation were performed by the iMovie software (Apple) to determine the (1) percentage of time the fly spent grooming, (2) the number of grooming bouts, and (3) the duration of individual grooming bouts. Groom bouts was considered complete when the fly stopped grooming by either remaining motionless or walking for more than 2 seconds. Flies were analysed at different time points during adulthood and $n \geq 15$ for each time point.

2.3 Statistical Analyses

The unpaired *t*-test was used to compare wild-type and mutant fly populations. All data are shown as mean ± S.E.M. Statistically significant comparisons are depicted as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.0001$, and $^{****}p < 0.0001$.

3 Results

hdc mutant flies are adult viable, but were reported to display defects in visual and mechanosensory behaviours (Burg et al., 1993; Melzig et al., 1996, 1998). Aiming at uncovering phenotypes that mimic those present in TS, an attempt at assessing the grooming behaviour of these flies and its variation with age was undertaken. It was hypothesised that based on recent work pointing to *HDC*

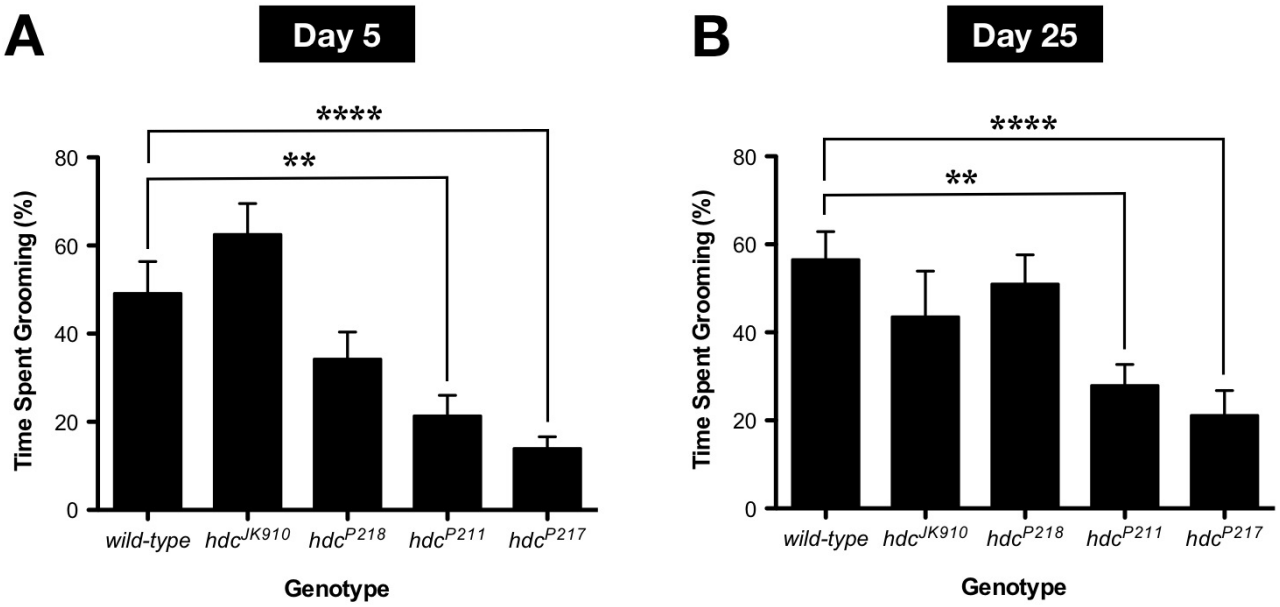

Figure 1: hdc^{P211} and hdc^{P217} mutant flies exhibit reduced grooming throughout adulthood. (A) At 5 days post-eclosion, hdc^{P211} and hdc^{P217} flies groom significantly less that control wild-type flies. This phenotype is not exhibited by hdc^{JK910} and hdc^{P218} flies. (B) A similar trend can be noted at 25 days post-eclosion. Data are represented as the mean ± S.E.M. percentage of time single male flies spend grooming during a 5 min observation period. $^{**}p < 0.01$, $^{****}p < 0.0001$, and $n \geq 15$ flies for each genotype at each time point.

as a TS susceptibility locus (Ercan-Sencicek et al., 2010; Karagiannidis et al., 2013), repetitive behaviours, and, hence, excessive grooming, are likely to be prominent in flies with homozygous loss-of-function *hdc* alleles.

To study baseline or spontaneous grooming behaviour, the activity of individual male flies was recorded in a small well-illuminated observation chamber. At 5 days old, hdc^{JK910} and hdc^{P218} flies groomed, on average, for 63% and 34% of the 5 min observation period, respectively. This result was not significantly different from that of controls, which were observed to spend about half (49%) of the time grooming. In contrast, age-matched hdc^{P211} and hdc^{P217} flies spent 21% and 14% of the observation window grooming, which was significantly lower than that of wild-type control flies ($p < 0.001$; Fig. 1A). This allelic-specific trend in grooming behaviour persisted with age, hence similar comparisons can be made at 25 days (Fig. 1B) and 35 days (data not shown) post-eclosion.

In addition to the total amount of time flies spent grooming, the duration and number of individual grooming bouts were assessed. At 5 and 25 days, the average duration of individual grooming bouts of both hdc^{JK910} and hdc^{P218} flies was found not to be statistically different from controls. Conversely, at these two time points, hdc^{P211} and hdc^{P217} flies had on average significantly shorter grooming bouts in comparison to wild-type counterparts (Fig. 2). Similar results were obtained at 35 days (data not shown). On investigating the number of grooming bouts occurring during the observation interval, hdc^{JK910} flies had a significantly lower number of grooming bouts at all time points measured. hdc^{P218} and hdc^{P217} flies only showed notable difference at day 25 whereas hdc^{P211} flies displayed no difference at all from control wild-type flies (Fig. 3).

In summation, our findings show that only the hdc^{P211} and hdc^{P217} alleles curb the amount of time dedicated to grooming as well as the duration of each grooming bout. However, the hdc^{JK910} and hdc^{P218} alleles exhibit a reduction in the number of grooming bouts. In this context, deficiency of histamine is associated with reduced rather than excessive spontaneous grooming activity in *Drosophila*.

4 Discussion

Animal models of human disease are key to identify genetic and, importantly, pharmacological modifiers of phenotypes that mimic those present in the human condition. In view of only a partial overlap with TS clinical features, *Hdc* (and *Slitrk1*) mouse knockouts might not be good models of this disorder (Cauchi & Tarnok, 2012). In the present study, we investigated whether *Drosophila* can serve as a TS animal model. To this end, we observed the grooming behaviour of adult flies at both an early and late stage during adulthood. In contrast to an earlier study, in which grooming was

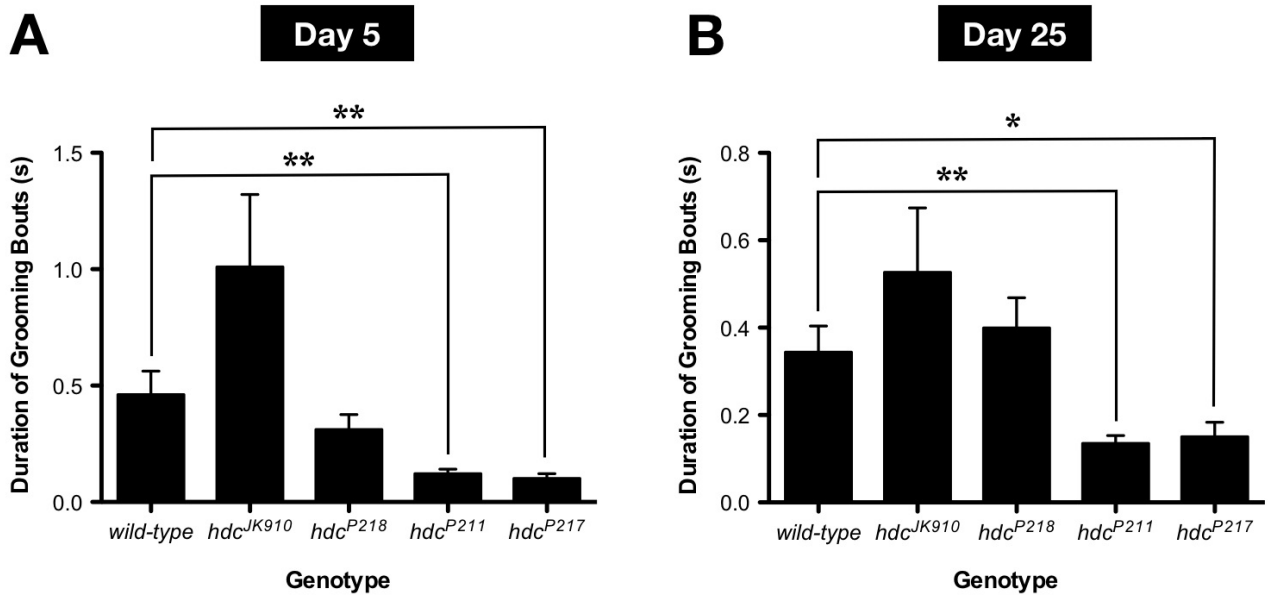

Figure 2: hdc^{P211} and hdc^{P217} mutant flies display a reduction in the duration of individual grooming bouts. At day 5 (A) and day 25 (B) of adulthood, the duration of individual grooming bouts is significantly shorter than controls. No change is however observed for hdc^{JK910} and hdc^{P218} mutant flies. Data are represented as the mean ± S.E.M. percentage of time single male flies spend grooming during a 5 min observation period. $^*p < 0.05$, $^{**}p < 0.01$, and $n \geq 15$ flies for each genotype at each time point.

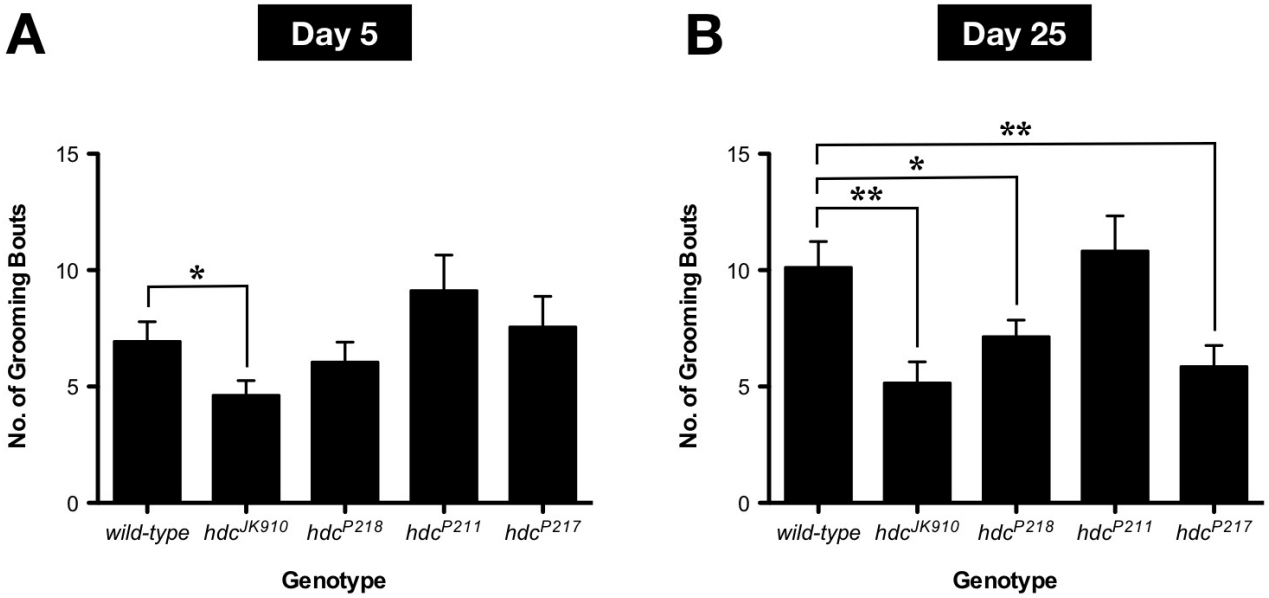

Figure 3: *Histamine deficiency reduces the number of grooming bouts.* (A) Day 5 hdc^{JK910} mutant flies exhibit a significant decrease in the number of grooming bouts. At this time point, the other *hdc* alleles analysed displayed no change compared to control. (B) At day 25, all *hdc* mutant flies except hdc^{P211} have a significant decrease in the number of grooming bouts. Data are represented as the mean ± S.E.M. percentage of time single male flies spend grooming during a 5 min observation period. $^*p < 0.05$, $^{**}p < 0.01$, and $n \geq 15$ flies for each genotype at each time point.

induced by coating flies with dust (Melzig et al., 1996), our study investigated baseline or spontaneous grooming. We hypothesised that spontaneous rather than prompted grooming, if repetitive, would closely mimic the persistent motor ticks that are a hallmark feature of TS. We find that histamine deficiency in *hdc* mutant flies does not induce an increase in spontaneous repetitive grooming behaviours but rather a decrease. Taking also into consideration the findings of Melzig et al. (1996), *hdc* mutants therefore have a reduction in both spontaneous (our study) as well as dust-induced grooming activity (Melzig et al., 1996).

Our results might imply that in humans, histaminergic neurons might have functions that are not present in *Drosophila*. Nonetheless, it is highly likely that the grooming behaviour in *Drosophila hdc* knockouts is not a translationally relevant TS phenotype. Future work should investigate whether repetitive grooming behaviour can be induced in the same mutants after pharmacological challenge or stress induction. This was found to be the case in mouse models. Hence, increased grooming and stereotypy were obvious in mouse *Hdc* knockouts only after amphetamine administration (Castellan Baldan et al., 2014) or following the stress induced by the presentation of a conditioned fear stimulus (Xu et al., 2015). These findings in *Hdc* knockout mice enhanced their validity as a pathophysiologically informative model of TS in addition to strengthening the value of the mouse model for drug discovery. Further investigations in fly *hdc* mutants with the aim of inducing stereotypy either by stress or drugs are therefore warranted. If successful, such studies can potentially validate the suitability of *Drosophila* as a novel animal model of TS.

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