nature neuroscience

A subset of octopaminergic neurons are important for *Drosophila* aggression

Chuan Zhou[1,2], Yong Rao[1] & Yi Rao[1,3]

Aggression is an innate behavior that is important for animal survival and evolution. We examined the molecular and cellular mechanisms underlying aggression in *Drosophila*. Reduction of the neurotransmitter octopamine, the insect equivalent of norepinephrine, decreased aggression in both males and females. Mutants lacking octopamine did not initiate fighting and did not fight other flies, although they still provoked other flies to fight themselves. Mutant males lost to the wild-type males in fighting and in competing for copulation with females. Enhanced octopaminergic signaling increased aggression in socially grouped flies, but not in socially isolated flies. We carried out genetic rescue experiments that revealed the functional importance of neuronal octopamine and identified a small subset of octopaminergic neurons in the suboesophageal ganglion as being important for aggression.

Although the general importance of aggression is obvious from the global to the individual levels, we find it surprising that biological research of aggression has not substantially increased over the last 30 years. Although it is not difficult to appreciate the negative consequences of excessive aggression, it is worth noting that aggression is an evolutionarily conserved behavior and a major force in both natural and sexual selections[1]. Aggression in the same species (intraspecific aggression) maximizes access to resources and increases the survival of the species. Animals are born with the capacity for aggression and individuals can display stereotypical agonistic patterns when isolated immediately after birth[2]. Genetic components influencing aggression have long been noticed and mutations affecting aggression are present in mammals[3,4].

Studies of aggression in *Drosophila*, one of the most powerful genetic models, began with an observation by Alfred Sturtevant, who discovered *Drosophila* aggression in 1915 (ref. 5). Those findings were buried in an article on sexual recognition, and there was no follow-up for four decades, with only one or two papers being published per decade from the 1950s to the 1970s that were related to fly aggression[6–8]. In 1960, a group characterized *Drosophila* aggression in much more detail and pioneered genetic studies of aggression[6]. In the 1980s, a series of ethological and laboratory studies of *Drosophila* aggression found that territorial successes were influenced by multiple factors such as age, body size, residency status, prior experience and geographic origin[9–14]. Aggression has now been described in both males and females[6,15,16], in sexually distinct manners controlled by genes regulating sexually specific behavior[17]. Heritable variations in aggression have been observed in *Drosophila*, indicating the feasibility of dissecting genetic components of *Drosophila* aggression. Artificial selection of flies with different levels of aggression followed by microarray analysis has led to the identification of differential genes[18,19].

Powerful tools that are available in *Drosophila* have facilitated neurobiological studies of aggression and have uncovered several genes that regulate different aspects of aggression. *fru* mutations affect courtship and recent studies found that *fru* mutants change the aggression pattern of males to that of females[17]. Serotonin and NPF regulate aggression in opposite directions[20]. In invertebrates, octopamine has been implicated in the regulation of aggression, with apparently different roles in different species[21–24]. In *Drosophila*, decreased aggression was observed in white-eyed $T\beta h^{nM18}$ mutants[25], which were defective in both vision and octopamine synthesis. One study reported a behavioral switch from aggression to courtship in *Tβh* mutant males[26]. Another found reduced lunging frequency in red-eyed *Tβh* mutants, *Tdc2* mutants and wild-type flies when their octopaminergic neurons were silenced[27].

There are approximately 70–100 octopaminergic neurons that are dispersed throughout the *Drosophila* nervous systems, regulating a spectrum of behaviors that include ovulation and learning[28,29]. Our genetic and behavioral studies have identified a small subset of neurons in aggression. Deletion of the gene encoding tyramine β hydroxylase (TβH), an enzyme involved in octopamine synthesis, decreased aggression without affecting locomotion, olfaction, sexual discrimination or courtship. Acute manipulations of octopaminergic neurons support a functional role for octopamine during aggression. Social interactions are important for regulating aggressive behavior in mammals as well as in flies; social isolation enhances aggression, whereas social grouping reduces aggression[12,30,31]. We have found that overexpression of TβH, the administration of an octopamine agonist or the transient activation of octopaminergic neurons increased aggression in socially grouped

[1]National Institute of Biological Sciences, Beijing, 7 Zhongguanchun Life Sciences Park, Beijing 102206, China. [2]Institute of Biophysics, Chinese Academy of Sciences, 15 Datun Rd., Chaoyang District, Beijing 100101, China. [3]School of Life Sciences and State Key Laboratory of Biomembrane, Peking University, 5 Yiheyuan Rd., Haidian District, Beijing 100871, China. Correspondence should be addressed to Yi Rao (yrao@pku.edu.cn).

Received 28 February; accepted 17 June; published online 1 August 2008; doi:10.1038/nn.2164

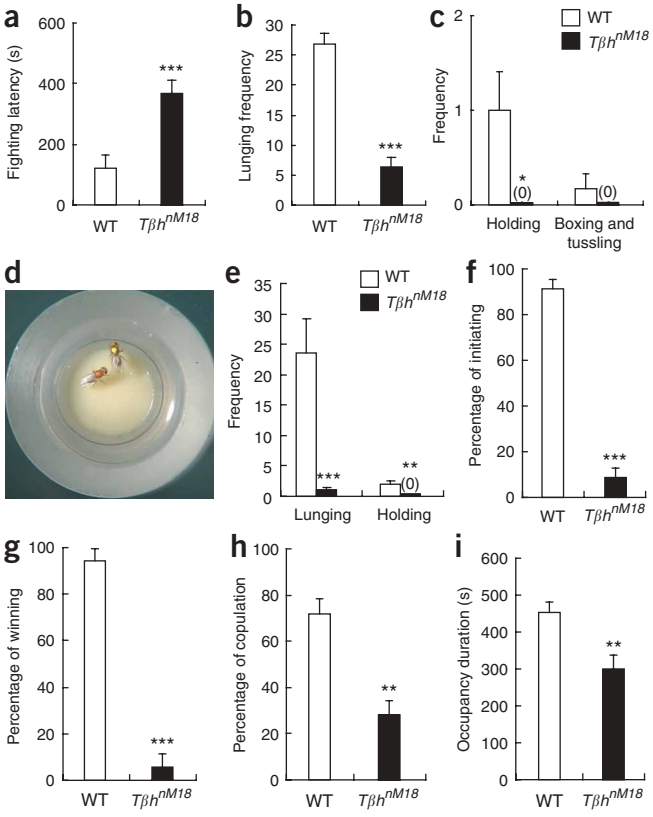

Figure 1 Effects of the *Tβh* mutation on male and female aggression. (**a**) Bar graph shows fighting latencies of $Tβh^{nM18}$ and wild-type (WT) flies. Fighting latencies were significantly longer in $Tβh^{nM18}$ mutant males than in the wild types (***$P < 0.001$, Mann Whitney test). (**b**) The lunging frequency was lower in $Tβh^{nM18}$ mutant males than in the wild types (***$P < 0.001$, Mann Whitney test). (**c**) Holding completely disappeared in $Tβh^{nM18}$ mutant males (*$P < 0.05$, Student's *t* test). (**d**) A $Tβh^{nM18}$ mutant male (marked with yellow) was paired with a wild-type male (marked with red). (**e**) $Tβh^{nM18}$ mutants showed significantly less lunging (***$P < 0.001$, Wilcoxon signed rank test) and holding than the wild types (**$P < 0.01$, paired *t* test). (**f**) Most of the agonistic encounters were initiated by the wild-type when paired with $Tβh^{nM18}$ mutants (***$P < 0.001$, Wilcoxon signed rank test). Flies initiating lunging, holding or boxing and tussling were considered to be initiators. (**g**) Wild-type flies won significantly more fights than $Tβh^{nM18}$ flies (***$P < 0.001$, Wilcoxon signed rank test). (**h**) The wild-type males mated more than the $Tβh^{nM18}$ males during mating competition (**$P < 0.01$, Wilcoxon signed rank test). (**i**) Wild-type males had significantly longer occupancy time than $Tβh^{nM18}$ mutant males (**$P < 0.01$, Wilcoxon signed rank test). Occupancy duration is the total time during the 10-min recording when a fly occupied the food patch. We tested 30 pairs for each group in **a**–**c**, 18 pairs in **e**–**g** and **i** and 50 pairs in **h**. Error bars represent s.e.m.

flies, but not in isolated flies. Octopamine is expressed in both neurons and non-neuronal cells outside of the brain. We found that aggression required neural octopamine. Genetic rescue experiments using the *UAS-Gal4* system in combination with a Gal80 suppressor revealed the functional importance of less than five octopaminergic neurons in the suboesophageal ganglion (SOG).

RESULTS

Reduced aggression in loss-of-function *TβH* mutants

We modified the assay for aggression that contains a food pad in the middle of a closed chamber (**Supplementary Fig. 1** online)[32]. The small size of the chamber increases the number of chances for contact and also makes it possible to videotape six chambers simultaneously. Two flies were placed into each chamber and videotaped. Analysis was carried out for the first 10 min after both flies were in the chamber. Latency of fighting was measured by the time from the placement of the flies into the chamber to the first fight; frequency was measured by the number of fights in a specific pattern in the first 10 min. Only fighting patterns involving physical engagement were scored in our studies and included lunging, holding, boxing and tussling[18,32]. Holding, boxing and tussling are collectively shown as HIF (high-intensity fighting).

It has been reported that aggression is reduced in flies that carry mutations in both the *white* gene and the *Tβh* gene, which encodes an important enzyme for octopamine synthesis[25]. A critical test was missing to distinguish whether the phenotype was the result of the *white* mutation or the $Tβh^{nM18}$ mutation. A previous study examined the fighting behavior of *white* mutants and observed an almost complete impairment in aggressive behavior of w^{1118} null mutants[27]. Consistent with these results, we found that the *white* mutants were defective in aggression (**Supplementary Fig. 2** online), as evidenced by lengthened fighting latency and reduced frequency of lunging and HIF. When *white* mutants were paired with wild-type flies, fights were initiated and won more by the wild-type flies than by the *white* mutants. These results indicate that *white* mutations affect aggression in the absence of mutations in *Tβh* and that the role of octopamine in aggression could not be established by experiments with flies carrying mutations for both *white* and *Tβh*.

To investigate the role of octopamine, we tested $Tβh^{nM18}$ mutant flies that had no mutation at the *white* locus. Octopamine was completely depleted in $Tβh^{nM18}$ mutants (**Supplementary Table 1** online). Fighting latency was significantly prolonged in $Tβh^{nM18}$ mutants ($P < 0.001$; **Fig. 1a**), whereas lunging and holding frequencies were reduced in $Tβh^{nM18}$ mutants ($P < 0.001$ and $P < 0.05$ for lunging and holding, respectively; **Fig. 1b,c**). The frequency of boxing and tussling was very low in the wild-type flies (**Fig. 1c**). Although $Tβh^{nM18}$ mutants had a decreased frequency of boxing and tussling, it was not significantly different from wild type ($P > 0.05$; **Fig. 1c**).

In *Drosophila*, both the levels and the patterns of aggression are different between males and females[16,17]. The most prominent pattern in female aggression is head butting, which is similar to lunging in males, except that females charge the opponents with the head rather than forelegs. We tested whether octopamine was also involved in female aggressive behavior. Pairs of females of the same genotype were tested. Fighting latency was prolonged in $Tβh^{nM18}$ mutant females, whereas head butting frequency was reduced (**Supplementary Fig. 3** online), indicating a common function of octopamine in male and female aggression.

Octopamine level is reduced in $Tβh^{nM18}$ mutants throughout development and adult life. To test whether octopamine is involved in aggressive behavior in adults, we manipulated the activity of octopaminergic neurons by using *Tdc2-Gal4* to drive the expression of $shibire^{ts}$ (shi^{ts}), a temperature-sensitive mutant of dynamin that can inhibit the vesicle recycling, thus blocking neural transmissions at the restrictive temperature[33]. Neither *Tdc2-Gal4* nor *UAS-*shi^{ts} alone affected aggression at 23 °C or 31 °C (**Supplementary Fig. 4** online). When both *Tdc2-Gal4* and *UAS-*shi^{ts} were present, aggression was reduced within 15 min of shifting to the restrictive temperature. Latency was longer at 31 °C than that at 23 °C (**Supplementary Fig. 4**), and frequencies of lunging and HIF were lower at 31 °C (**Supplementary Fig. 4**). Consistent with previous results[27], our

finding that acutely silencing octopaminergic neurons phenocopies the $T\beta h^{nM18}$ mutant indicates that the aggression phenotype in $T\beta h^{nM18}$ mutants is not a result of developmental defects.

Reduced aggression between the $T\beta h^{nM18}$ mutants could mean that a mutant fly either does not initiate aggression against another fly or does not elicit aggression by others. To distinguish between these possibilities, we paired $T\beta h^{nM18}$ mutants with wild-type flies (**Fig. 1d** and **Supplementary Video 1** online). Wild-type flies still fought the $T\beta h^{nM18}$ mutants, whereas the mutants did not initiate fighting and did not fight back (**Fig. 1e,f**). These results indicate that the mutants do not fight, but are still able to elicit fighting by others.

Aggression is important for the resource holding power of mammals. In flies, a study with six wild-type males found a correlation between aggressiveness and mating success[7], but *ebony* males were found to have reduced mating with enhanced territorial aggression[6,8]. To determine whether and how the resource holding power was affected in $T\beta h^{nM18}$ mutants, we tested their ability to compete for females and food. When placed in the fighting chamber with food in the middle, wild-type flies won the majority of fights (**Fig. 1g**). When a mutant male and a wild-type male were placed in the same chamber with a wild-type virgin female, the chance for copulation was significantly lower for the $T\beta h^{nM18}$ mutant than for the wild type ($P < 0.01$; **Fig. 1h**). The total time on the flies spent on the central patch of food in the fighting chamber was measured as the occupancy duration. $T\beta h^{nM18}$ mutants spent significantly less time than the wild-type flies on the food pad ($P < 0.01$; **Fig. 1i**). Our results indicate that $T\beta h^{nM18}$ mutants are less successful than wild-type in their competition for resources and for females.

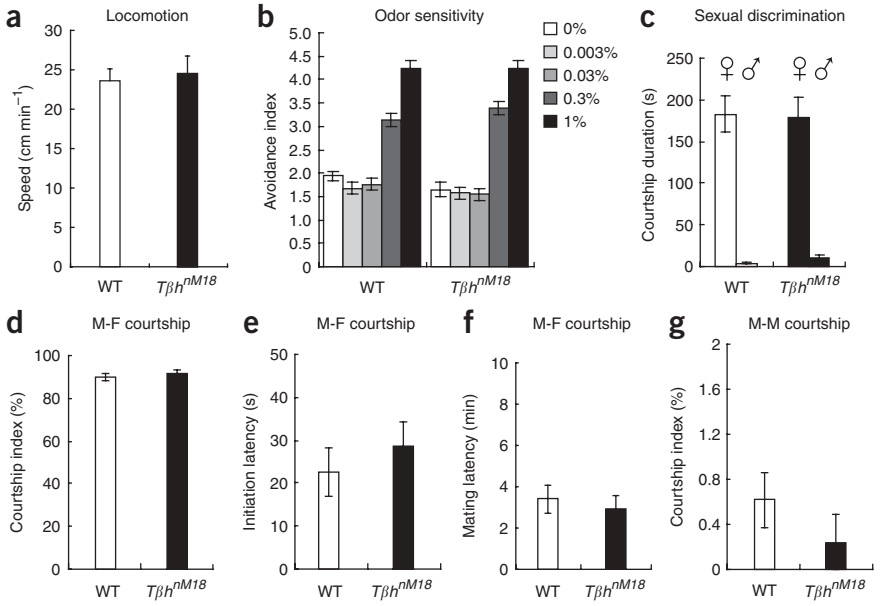

Figure 2 General behavioral characterization of $T\beta h^{nM18}$ mutants. (**a**) Locomotive activities were measured by the speed of a single fly over a 4-min period ($n = 24$ for each group). (**b**) Avoidance indices of $T\beta h^{nM18}$ and control males in an odor-sensitivity assay ($n = 50$ for each group). (**c**) $T\beta h^{nM18}$ mutant and wild-type males behaved similarly in showing sexual preference toward decapitated females over decapitated males ($n = 23$ for the control group, $n = 24$ for the mutant group). (**d**) Male-female (M-F) courtship indices of $T\beta h^{nM18}$ and control males courting wild-type virgin females ($n = 22$ for the wild-type group, $n = 24$ for the mutant group). (**e**) Initiation latency of $T\beta h^{nM18}$ and control males. $T\beta h^{nM18}$ and wild-type males were not significantly different in initiating courtship toward wild-type virgin females ($n = 22$ for the wild-type group, $n = 24$ for the mutant group). (**f**) $T\beta h^{nM18}$ and control males had similar mating latencies ($n = 23$ for each group). (**g**) Male-male (M-M) courtship indices of $T\beta h^{nM18}$ and control males were not significantly different ($n = 20$ for the wild-type group, $n = 18$ for the mutant group). $P > 0.05$ for all comparisons above, Mann Whitney test. All values are mean ± s.e.m.

Behavior patterns were unaffected in $T\beta h^{nM18}$ mutants

The decreased aggression of $T\beta h^{nM18}$ could result from a general defect in movement, an inability of male flies to find their usual opponents or a defect in motivation for any action. To test whether the aggression phenotype of $T\beta h^{nM18}$ is an indirect result of other behavioral defects, we first assessed locomotion by measuring the speed of individual flies in a round chamber. No difference was found between $T\beta h^{nM18}$ mutants and wild-type flies (**Fig. 2a**). $T\beta h^{nM18}$ mutants were then tested for their ability to sense and avoid specific odorants. The avoidance index of $T\beta h^{nM18}$ mutants to benzaldehyde did not differ significantly from that of the wild type ($P > 0.05$; **Fig. 2b**), indicating that there was no defect in the odor sensitivity and the avoidance behavior in $T\beta h^{nM18}$ mutants.

To test the ability of flies to distinguish between males and females, we placed a decapitated wild-type female and a decapitated wild-type male in two opposite ends of a chamber that contained a $T\beta h^{nM18}$ mutant or wild-type male. $T\beta h^{nM18}$ males were similar to the wild-type males in their preference for spending more time with the females, showing little interest in the males (**Fig. 2c**), which suggests that reduced aggression in $T\beta h^{nM18}$ mutants was not a result of an inability to distinguish the sexual identities of opponents.

We tested whether courtship behavior was affected in $T\beta h^{nM18}$ mutants. In the typical male-female courtship assay[34], $T\beta h^{nM18}$ and wild-type males had similar courtship indices, initiation latencies and mating latencies (**Fig. 2d–f**). Reduced aggression between males could also be a consequence of increased male-male courtship behavior. When tested with a male-male courtship assay, $T\beta h^{nM18}$ males were similar to wild-type males in that neither showed significant male-male courtship ($P > 0.05$; **Fig. 2g**).

It has recently been reported that octopamine is required for making a choice between aggression and courtship, a conclusion that is based on an analysis of the behavioral patterns of $T\beta h^{nM18}$ mutants after a male fly showed wing extension[26]. In contrast, we found a general decrease of aggression, but no significant increase of courtship in $T\beta h^{nM18}$ mutants ($P > 0.05$; **Fig. 2g**). The frequency of unilateral wing vibration, which is an early step in courtship, was indistinguishable between $T\beta h^{nM18}$ mutants and wild types (**Supplementary Fig. 5** online), whereas the frequency of wing threat (bilateral wing extension), a typical step in aggression, was significantly reduced in $T\beta h^{nM18}$ mutants ($P < 0.001$; **Supplementary Fig. 5**). It is possible that the previous study might have mixed wing extensions of different kinds (such as the unilateral wing vibration and bilateral wing threat) and interpreted a simple reduction of aggression as a concomitant reduction of aggression and increase of courtship, or, alternatively, different setups for measuring aggression might have contributed to the discrepancies of behavior outputs.

Our results suggest that the aggression phenotype of $T\beta h^{nM18}$ mutants is not secondary to defects in general behavior patterns.

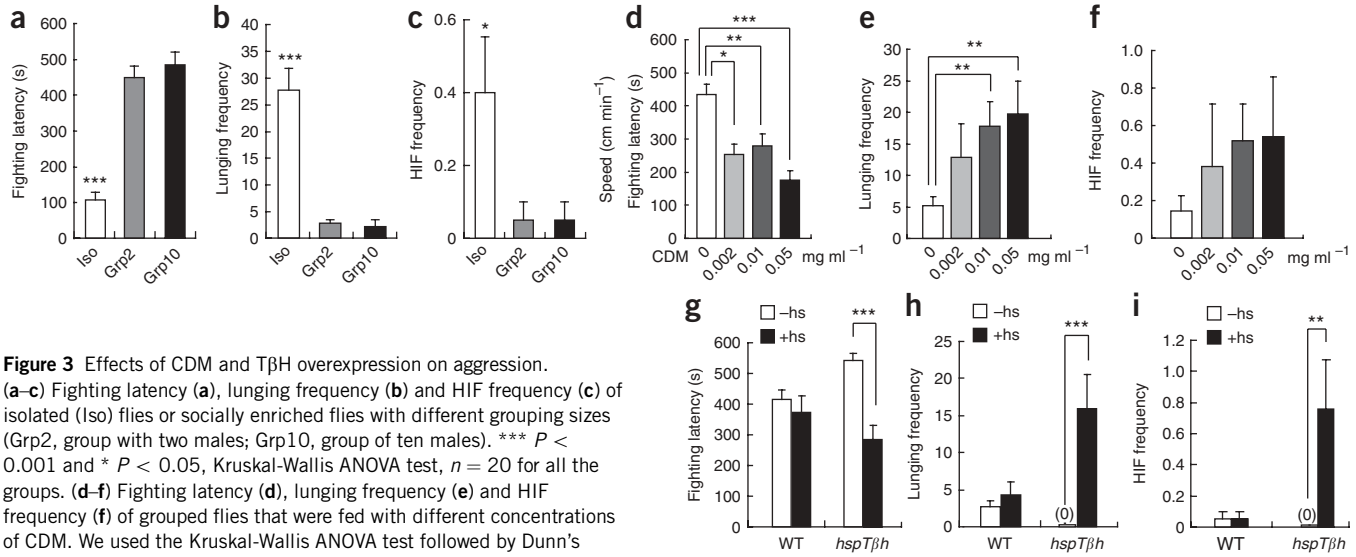

Figure 3 Effects of CDM and TβH overexpression on aggression. (**a**–**c**) Fighting latency (**a**), lunging frequency (**b**) and HIF frequency (**c**) of isolated (Iso) flies or socially enriched flies with different grouping sizes (Grp2, group with two males; Grp10, group of ten males). *** $P <$ 0.001 and * $P < 0.05$, Kruskal-Wallis ANOVA test, $n = 20$ for all the groups. (**d**–**f**) Fighting latency (**d**), lunging frequency (**e**) and HIF frequency (**f**) of grouped flies that were fed with different concentrations of CDM. We used the Kruskal-Wallis ANOVA test followed by Dunn's multiple comparison test to identify significant differences. We found that 0.002 mg ml^{-1} CDM was sufficient to change fighting latency (*$P < 0.05$), whereas 0.01 mg ml^{-1} CDM significantly increased the lunging frequency of socially enriched flies (**$P < 0.01$). All values are mean ± s.e.m (0 mg ml^{-1}, $n = 28$; 0.002 mg ml^{-1}, $n = 21$; 0.01 mg ml^{-1}, $n = 29$; 0.05 mg ml^{-1}, $n = 24$). (**g**–**i**) Heat-shocked *hspTβh* flies had shortened fighting latency (**g**) and increased lunging frequency (**h**). ***$P < 0.001$, Mann Whitney test. HIF frequency (**i**) was also increased in heat-shocked *hspTβh* flies (** $P < 0.01$). Heat shock treatment (+hs indicates heat shock, –hs indicates no heat shock) did not affect aggression in wild-type flies ($P > 0.05$). We tested 25 pairs of *hspTβh* flies and 20 pairs of wild-type flies. Error bars represent s.e.m.

Enhanced octopaminergic signaling increases aggression

Social experience is important for the development of aggressive behavior in both mammals and insects, with socially isolated males fighting more than those that were raised in groups. In our studies, male flies were raised in isolation or in groups of two or ten flies of the same age until day 5. Flies with identical rearing conditions were paired and tested. As shown previously[12], grouping markedly reduced aggression. Furthermore, we found that a group of two flies was as effective as a group of ten flies at reducing aggression (**Fig. 3a–c**).

To address the question of whether enhancing octopamine signaling could restore aggressiveness in socially grouped flies, we first tested the effects of chlorodimeform (CDM) on fly aggression. Notably, treatment of grouped flies with CDM, an octopamine agonist, reduced the fighting latency (**Fig. 3d**) and increased the lunging frequency (**Fig. 3e**) without significantly affecting the HIF frequency ($P > 0.05$; **Fig. 3f**). Moreover, TβH was overexpressed in grouped flies by a TβH transgene under the control of the heat shock promoter (*hspTβh*)[28]. After heat shock induction for 30 min, flies were allowed to recover for 3 h. *hspTβh* flies had shortened fighting latency (**Fig. 3g**), increased lunging frequency (**Fig. 3h**) and increased HIF frequency (**Fig. 3i**). Heat shock for the same amount of time did not affect aggression in wild-type controls (**Fig. 3g–i**).

We further used the *UAS-NaChBac*/*Tub-Gal80^{ts}* system to activate octopaminergic neurons and examined its effect on aggression. At the permissive temperature, Gal80^{ts} binds to and inhibits the transcription activation activity of Gal4. When shifted to the restrictive temperature, Gal80^{ts} becomes nonfunctional, allowing Gal4 to activate the transcription of NaChBac, a bacterially derived voltage-sensitive sodium channel with a lower threshold for activation and slower kinetics for inactivation compared with voltage-sensitive sodium channels in flies, which causes neuronal activation in *Drosophila*[35]. The tubulin (Tub) *αTub84b* promoter drives the expression of Gal80^{ts} in all cells. Combining *UAS-NaChBac* and *Tub-Gal80^{ts}* with *Tdc2-Gal4* allowed us to express NaChBac in adult octopaminergic neurons, thus activating them post-developmentally (**Fig. 4a**). *Tdc2-Gal4*, *UAS-NaChBac* or *Tub-Gal80^{ts}* alone did not affect aggression (**Fig. 4b–d**). When all three components were present, aggression in grouped flies was increased after shifting to the restrictive temperature, with shorter latency and higher lunging and HIF frequencies (**Fig. 4b–d**), indicating that the activation of octopaminergic neurons reversed the reduction of aggression by social grouping of flies.

We also asked whether enhancing octopamine signaling could promote aggression in socially naive flies. We did not detect any significant effect of either CDM treatment or overexpression of TβH by heat shock–inducible promoter in flies that were raised in social isolation ($P > 0.05$; **Supplementary Fig. 6** online). Two possibilities can explain this phenomenon. The first possibility is that octopamine is involved in resetting aggression after social experience. The second possibility is that the level of aggressiveness is saturated in socially naive flies, which occludes further enhancement of aggression by other treatments. The first possibility would be further supported if the octopamine concentration is changed by social experience. We thus examined the concentrations of bioamines in the brains of males by high-performance liquid chromatography with electrochemical detection, but detected no difference in octopamine level between grouped and isolated flies (**Supplementary Table 1**). This does not completely rule out the first possibility, as the concentration of octopamine in a limited number of neurons could be changed, but this could not be detected when whole heads were assayed.

Neural TβH expression restores aggression in *TβH* mutants

Octopamine exists both inside and outside of the nervous system, functioning as either a neurotransmitter or a hormone in insects[36]. It is therefore important to investigate whether the aggression phenotype of *Tβh* mutants was a result of defects in the nervous system.

In *Drosophila*, two enzymes, dTdc1 and dTdc2, are involved in the first step of octopamine synthesis, with dTdc1 being expressed outside of the brain and dTdc2 being expressed in the brain[36]. We confirmed

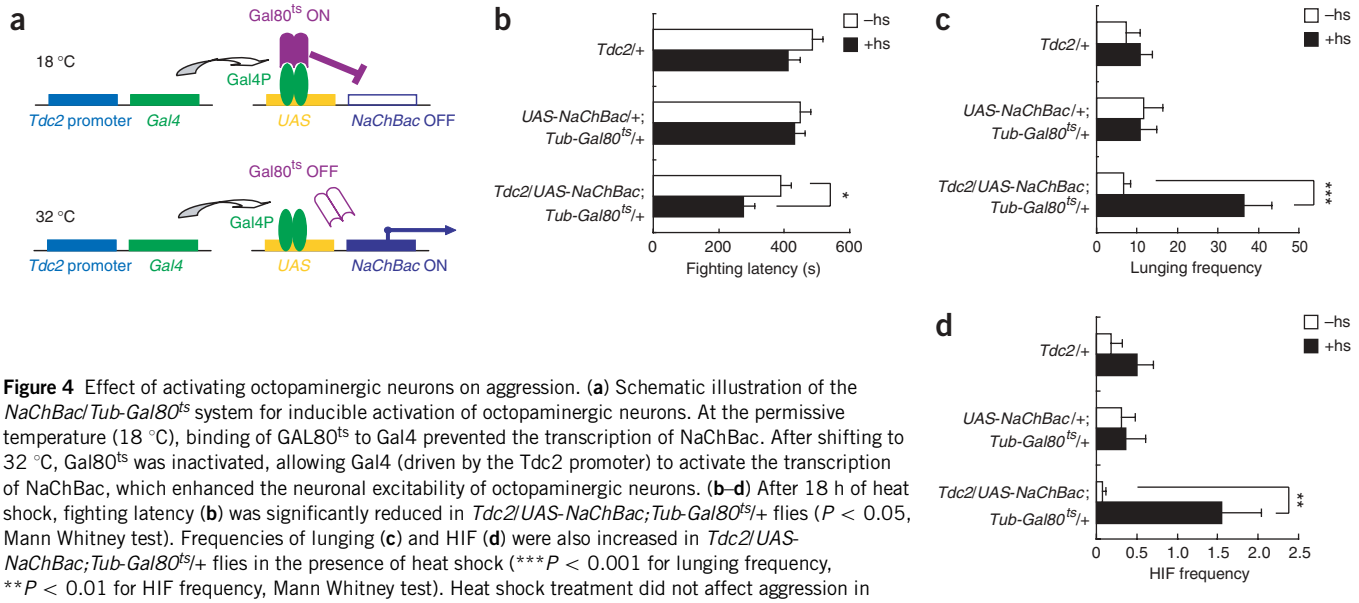

Figure 4 Effect of activating octopaminergic neurons on aggression. (**a**) Schematic illustration of the *NaChBac/Tub-Gal80^{ts}* system for inducible activation of octopaminergic neurons. At the permissive temperature (18 °C), binding of GAL80^{ts} to Gal4 prevented the transcription of NaChBac. After shifting to 32 °C, Gal80^{ts} was inactivated, allowing Gal4 (driven by the Tdc2 promoter) to activate the transcription of NaChBac, which enhanced the neuronal excitability of octopaminergic neurons. (**b**–**d**) After 18 h of heat shock, fighting latency (**b**) was significantly reduced in *Tdc2/UAS-NaChBac;Tub-Gal80^{ts}/+* flies ($P < 0.05$, Mann Whitney test). Frequencies of lunging (**c**) and HIF (**d**) were also increased in *Tdc2/UAS-NaChBac;Tub-Gal80^{ts}/+* flies in the presence of heat shock (***$P < 0.001$ for lunging frequency, **$P < 0.01$ for HIF frequency, Mann Whitney test). Heat shock treatment did not affect aggression in either *Tdc2/+* or *UAS-NaChBac/+; Tub-Gal80^{ts}/+* flies ($P > 0.05$, Mann Whitney test). Error bars represent s.e.m. (number of pairs tested in each group: $n = 28$ or 28 for *Tdc2/+* with or without heat shock, $n = 33$ or 32 for *UAS-NaChBac/+;Tub-Gal80^{ts}/+* with or without heat shock, $n = 40$ or 41 for *Tdc2/UAS-NaChBac;Tub-Gal80^{ts}/+* with or without heat shock, respectively).

the expression pattern of *Tdc2-Gal4* and *Tdc1-Gal4* by staining the adult male brains of *Tdc2-Gal4/UAS-mCD8:GFP* and *Tdc1-Gal4/UAS-mCD8:GFP* with antibody to Tβh (**Fig. 5a–h** and data not shown). Consistent with previous reports, GFP expression was not detected in the brain of *Tdc1-Gal4/UAS-mCD8:GFP* heterozygous flies (data not shown). In *Tdc2-Gal4/UAS-mCD8:GFP* flies, only two populations of octopaminergic neurons, one in the antenna lobe and another in the SOG, were positive for both GFP and TβH immunoreactivity (**Fig. 5a–h**). *Tdc2-Gal4* was unable to drive expression in octopaminergic neurons in other brain regions, such as the protocerebrum, the fan-shaped body and the central complex (**Fig. 5b**).

To functionally differentiate the neuronal and endocrine contribution of octopamine, we used *Tdc2-Gal4* and *Tdc1-Gal4* to drive *UAS-Tβh* in *Tβh^{nM18}* mutant males. *Tdc2-Gal4*, *Tdc1-Gal4* or *UAS-Tβh* alone did not affect aggression in *Tβh^{nM18}* mutant males. The combination of *Tdc2-Gal4* and *UAS-Tβh* rescued aggression deficiency in *Tβh^{nM18}* males: the latency was shortened (**Fig. 5i**) and the frequencies for both lunging and HIF were increased (**Fig. 5j,k**). *Tβh* expression, driven by *Tdc2-Gal4*, could also effectively rescue the aggression phenotype of female *Tβh^{nM18}* mutants (**Supplementary Fig. 3**). In contrast, *Tdc1-Gal4*–driven *TβH* expression failed to rescue the aggression phenotype of *Tβh^{nM18}* mutants with regard to fighting

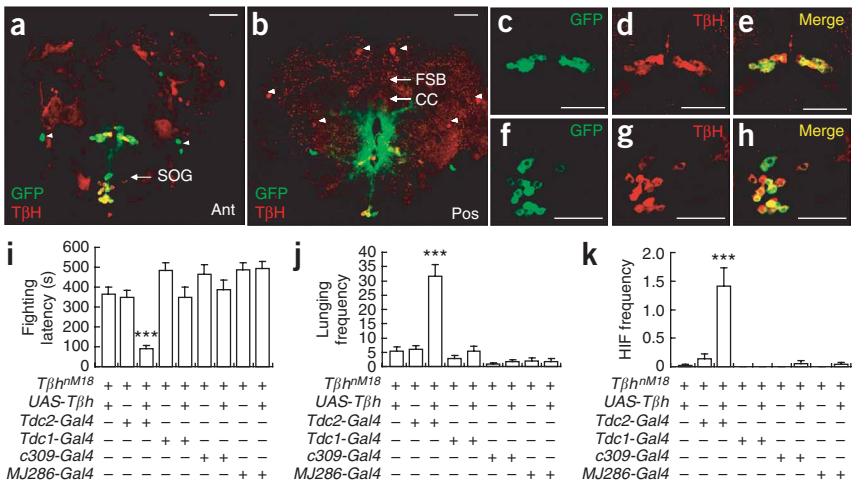

Figure 5 Rescue of aggression phenotype in *Tβh^{nM18}* mutants by TβH expression in octopaminergic neurons. (**a**) Anterior confocal sections of a *Tdc2-Gal4/UAS-mCD8:GFP* adult male brain stained with antibody to TβH (red). Arrowheads point to neurons that are negative for TβH and positive for GFP: non-octopaminergic neurons in which *Tdc2-Gal4* could still drive gene expression. (**b**) Posterior confocal sections of a *Tdc2-Gal4/UAS-mCD8:GFP* adult male brain stained with the antibody to TβH (red). TβH immunostainings were evident in the protocerebrum, the fan-shaped body (FSB) and the central complex (CC), whereas *Tdc2-Gal4* could not drive GFP expression (green) in these neurons (indicated by the arrowhead). (**c**–**e**) Higher magnification views of a population of *Tdc2-Gal4*–positive neurons in the antennal lobe (AL). (**f**–**h**) Higher magnification images of a cluster of *Tdc2-Gal4*–positive neurons in the SOG. In **c**–**h**, GFP expression is viewed in green and antibody to TβH staining is red. Scale bars in **a**–**h** represent 50 μm. (**i**) The fighting latency in *Tβh^{nM18}* mutant males was reduced when both *Tdc2-Gal4* and *UAS-Tβh* were introduced, but not when only one of the transgenes was present (***$P < 0.001$, Kruskal-Wallis ANOVA test). In contrast, expression of TβH under the control of TDC1 promoter did not rescue *Tβh^{nM18}* phenotype ($P > 0.05$). Similarly, expressing TβH by *c309-Gal4* or *MJ286-Gal4* failed to increase the aggression level of *Tβh^{nM18}* mutants ($P > 0.05$). $n = 46$, 49, 51, 20, 18, 18, 18, 27 and 26, respectively. (**j**) Lunging frequency of indicated genotypes (***$P < 0.001$, Kruskal-Wallis ANOVA test). (**k**) The frequency of HIF in indicated strains (***$P < 0.001$, Kruskal-Wallis ANOVA test). All values are mean ± s.e.m.

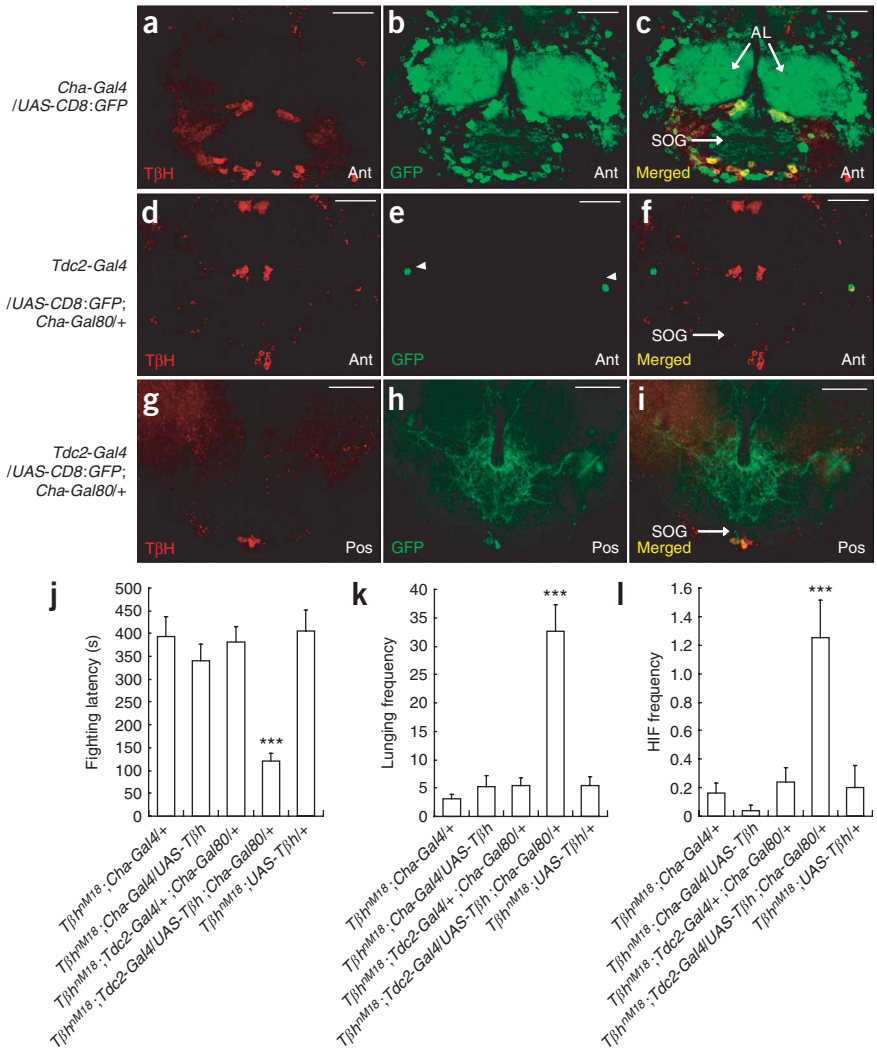

Figure 6 A small subset of octopaminergic neurons involved in aggression. (**a**–**c**) Confocal sections of a *Cha-Gal4*/*UAS-mCD8:GFP* (green) adult male brain stained with antibody to TβH (red). Note that most of the octopaminergic neurons in the antennal lobe or the SOG showed GFP expression. Scale bars represent 50 μm. (**d**–**f**) Anterior confocal sections of a *Tdc2-Gal4*/*Cha-Gal80*/*UAS-mCD8:GFP* (green) adult male brain stained with antibody to TβH (red).The Gal4 activity of Tdc2 driver was markedly suppressed. Note that the GFP-positive neurons (green, indicated by arrowheads) in **e** could not be stained with the antibody to TβH and are thus not octopaminergic neurons. Scale bars represent 50 μm. (**g**–**i**) Posterior confocal sections of a *Tdc2-Gal4*/*Cha-Gal80*/*UAS-mCD8:GFP* (green) adult male brain stained with antibody to TβH (red). The Gal4 activity of Tdc2 driver was markedly suppressed, except for two neurons in the SOG which were positive for the TβH staining, but were negative for GFP. The number of neurons with this property varied from 2 to 5 in different animals. Scale bars represent 50 μm. (**j**) Fighting latency of indicated genotypes. TβH expression driven by *Tdc2-Gal4* in the presence of the *Cha-GalL80* repressor could still rescue the $T\beta h^{nM18}$ phenotype. (**k**) Lunging frequency of indicated genotypes. (**l**) HIF frequency of indicated genotypes. $^{***}P < 0.001$. All values are mean ± s.e.m. Statistical analyses were carried out by nonparametric Kruskal-Wallis ANOVA test.

latency, lunging frequency and HIF frequency, arguing against the involvement of hormonal octopamine in aggression (**Fig. 5i–k**).

We also used other Gal4 lines to express TβH in different brain regions of $T\beta h^{nM18}$ mutants in an attempt to find the location of the subset of octopaminergic neurons involved in aggression. The $T\beta h^{nM18}$ mutant phenotype could not be rescued by TβH expression under the control of either *c309-Gal4*, which drives expression in the mushroom bodies, or *MJ286-Gal4*, which drives expression in a cluster of neurons in lateral protocerebrum (**Fig. 5**). Therefore, we conclude that the aggression phenotype in $T\beta h^{nM18}$ mutants is the result of a TβH deficiency in the nervous system.

A subset of octopaminergic neurons involved in aggression

To further define the subset of octopaminergic neurons that is involved in aggression, we made use of the drivers *Cha-Gal4* and *Cha-Gal80*. These drivers were made by fusing Gal4 or Gal80 to the promoter of choline acetyltransferase (Cha), and they drive expression in cholinergic neurons[37] (**Fig. 6**).

As we have shown that the antennal lobe and the SOG populations of octopaminergic neurons positive for *Tdc2-Gal4* were involved in aggression, we checked whether these neurons overlapped with neurons in which *Cha-Gal4* could drive gene expression. All of the antennal lobe and most of the SOG neurons that were immunoreactive to antibody to TβH were also positive for GFP driven by *Cha-Gal4* (**Fig. 6a–c**). Behaviorally, we found that *Cha-Gal4*–driven TβH expression could not rescue the aggression phenotype in $T\beta h^{nM18}$ mutants (**Fig. 6j–l**). These results suggest that neurons that were positive for TβH and *Cha-Gal4* are not responsible for the aggression deficit in $T\beta h^{nM18}$ mutants.

Cha-Gal80 allowed us to carry out the complementary experiment: asking the importance of neurons that are positive for octopamine, but negative for *Cha*-driven gene expression. We used the *Tdc2-Gal4* driver to express GFP in the presence of *Cha-Gal80* and found that GFP expression in all of the antennal lobe neurons and most of the SOG neurons was suppressed, with only 2–5 SOG octopaminergic neurons being GFP positive (**Fig. 6d–i**). Therefore, we could use the combination of *Tdc2-Gal4* and *Cha-Gal80* to drive TβH expression in those SOG neurons. We found that TβH expression that was driven by *Tdc2-Gal4* and *Cha-Gal80* could rescue the aggression phenotype in $T\beta h^{nM18}$ mutants (**Fig. 6j–l**). These results indicate that a distinct subset of octopaminergic neurons in the SOG is functionally important for aggression.

DISCUSSION

Both loss-of-function and gain-of-function studies support the idea that octopamine in the adult brain is involved in *Drosophila* aggression. Our genetic and behavioral studies lead us to conclude that a subset of octopaminergic neurons located in the SOG is important for aggression in flies. This conclusion is based on several results. First, our experiments using the *Tdc2-Gal4* driver suggest that octopaminergic neurons in the antennal lobe and the SOG are involved in aggression. *Tdc2-Gal4*–driven GFP expression can only partially recapitulate the octopaminergic circuits in the brain, with some of the GFP-positive neurons

lacking endogenous TβH. Only those in the antennal lobe and the SOG were found to express endogenous TβH. This finding, when considered with the fact that exogenous TβH that was driven by *Tdc2-Gal4* could rescue the aggression phenotype in $T\beta h^{nM18}$ mutants, suggests that the antennal lobe and SOG octopaminergic neurons are involved in aggression. Second, the failure of TβH that was driven by *Cha-Gal4* to rescue $T\beta h^{nM18}$ mutant phenotype indicates that octopaminergic neurons, in which *Cha-Gal4* could drive gene expression, are not responsible for the $T\beta h^{nM18}$ phenotype. Third, results from our experiments using *Cha-Gal80* show that less than five octopaminergic neurons in the SOG are important for aggression. GFP expression driven by *Tdc2-Gal4* was suppressed by *Cha-Gal80* in most neurons, with the exception of 2–5 octopaminergic neurons in the SOG. Thus, the ability of TβH, driven by *Tdc2-Gal4* in the presence of *Cha-Gal80*, to rescue the $T\beta h^{nM18}$ mutant phenotype indicates that the 2–5 octopaminergic neurons in the SOG are sufficient to rescue the aggression phenotype in $T\beta h^{nM18}$ mutants, demonstrating the functional importance of this small subset of octopaminergic neurons.

Social isolation markedly affects both aggression and sleep in flies[31,38]. Our results indicate that treatment with the octopamine agonist CDM, overexpression of TβH or activation of octopaminergic neurons with NaChBac can increase aggression in grouped flies, but not in socially naive flies. These results could be interpreted as occlusion: the level of aggression in socially isolated flies is already very high and could not be further increased by other treatments. They could also be interpreted as supporting the idea that octopamine is involved in the social regulation of aggression. We examined the concentration of octopamine in the heads of grouped and isolated flies and could not detect substantial changes in octopamine levels being caused by social grouping. This neither supports nor disproves a role for octopamine in social experience. It is possible that the level of octopamine is changed by social isolation in only a few neurons, which is undetectable when whole heads are measured. It is also possible that the sensitivity of postsynaptic neurons for octopamine or the activity of octopamergic neurons, but not the synthesis of octopamine, are changed by social experience. Further work is required to determine the role of octopamine in social interactions.

Our results support the notion that the aggression phenotype in *Tβh* mutants was not a secondary result of changes in sexual discrimination or sexual behavior. Our results have shown that *Tβh* mutants display normal sexually related activities. The conclusion of a recent report of octopamine involvement in making a choice between aggression and courtship[26] is not supported by our results, probably because we used different experimental setups or because the previous report did not separately analyze unilateral wing extension (a part of courtship) and bilateral wing extension (the wing threat in aggression), indicating that a simple reduction of aggression might have thus been interpreted as an increase in courtship. When we separately counted unilateral and bilateral wing extension, we indeed found an expected decrease of bilateral wing extension in *Tβh* mutants, but found no change in unilateral wing extension. Consistent with our results, a previous study also observed generally decreased aggression behavior in *Tβh* mutants[27], although they did not examine the role of octopamine in courtship.

The roles of octopamine and norepinephrine in other species have been studied, although not to the extent that is possible in flies. In invertebrates, the roles of octopamine in aggression seem to vary between species. Octopamine injection into the haemolymph of lobsters leads to postures with extended limbs and abdomens, resembling the posture of subordinates[21,39], whereas serotonin injection produces rigidly flexural legs that are highly aggressive, indicating opposite roles of octopamine and serotonin in lobster aggression[21,22]. In crickets, the level of octopamine in haemolymph was increased during fighting as well as during flight[40]. Chemical depletion of both octopamine and dopamine led to reduced aggression and locomotion in crickets[23]. Crickets that have previously lost fights learn to retreat when tested again with winners[24]. However, injection of an octopamine receptor agonist could reverse such learned retreats, although it did not affect aggression in naive crickets[24]. In flies, enhanced octopamine signaling could not affect the establishment of dominance during fighting (**Supplementary Fig. 7** online).

Octopamine is the insect counterpart of norepinephrine and its receptors in insects are homologous to mammalian adrenoceptors[41]. The best evidence for norepinephrine involvement in aggression was provided by mice that lacked the gene for the α2c-adrenergic receptor[42], an autoinhibitory receptor. Mice without this receptor display increased aggressive behavior, whereas α2c overexpression decreased aggression[42]. In *Dbh* knockout mice completely lacking noradrenaline, the aggressive response is essentially eliminated in a resident-intruder protocol[43]. One important question in the future should be whether and how norepinephrine and its receptors in defined locations of the mammalian brain are involved in aggression.

METHODS

Stocks and rearing conditions. Flies were usually reared at 25 °C (or 18 °C for flies involving *UAS-shi^{ts}* or *Tub-Gal80^{ts}*) and 60% humidity and were kept in a 12:12-h light-dark circle. Behavioral assays were carried out at 25 °C and 60% humidity between 15:00 and 19:00. Because the original $T\beta h^{nM18}$ mutants were white-eyed, we backcrossed the flies into a Canton S strain to generate recombinant w^+,$T\beta h^{nM18}$ lines. Nonrecombinant w^+ flies served as the wild-type controls. In all experiments, Canton S flies were used as controls, with the exception of experiments involving *UAS-shi^{ts}* and *UAS-NaChBac*/*Tub-Gal80^{ts}*, in which Gal4 or UAS flies were crossed with the w^{1118} strain to generate heterozygous controls. The $T\beta h^{nM18}$ deletion mutant was a gift from C.-F. Wu (University of Iowa). *hspTβh* and *UAS-Tβh* were generously provided by M. Monastirioti (Institute of Molecular Biology and Biotechnology, Greece). *Tdc2-Gal4* was a gift from J. Hirsh (University of Virginia). *UAS-shi^{ts}* was a gift from P. Shen (University of Georgia). *UAS*-NaChBac was a gift from B. White (US National Institute of Mental Health). MJ286-*Gal4* was a gift from R. Greenspan (The Neuroscience Institute). C309-*Gal4*, *Cha-Gal4* and *Cha-Gal80* were generously provided by P. Salvaterra and T. Kitamoto (Beckman Research Institute of the City of Hope). *Tub-Gal80^{ts}* was obtained from the Bloomington Stock Center.

Immunohistochemistry. The antibody to TβH was generated by immunizing rabbit with purified his-tag protein containing the whole TβH protein sequence. For all the immunostainings, 5–10-d-old adult male flies were collected after eclosion and the CNS was dissected in ice-cold PBS. The adult brains were subject to 4% paraformaldehyde (wt/vol) fixation in 0.3% PBT (phosphate-buffered saline containing 0.3% Triton-X100 (vol/vol)) for 30 min and subsequently washed three times with 0.3% PBT for 15 min at 22–27 °C. Samples were transferred to 5% normal goat serum (vol/vol, diluted in 0.3% PBT) for 1 h blocking at room temperature and incubated with antibody to TβH (1:1,000, diluted in 5% normal goat serum) at 4 °C overnight. After washing samples three times for 15 min with 0.3% PBT at room temperature and incubating samples in secondary antibody (Molecular Probes, 1:500) for 2 h at 37 °C, samples were mounted in 70% glycerol and imaged on a Zeiss LSM5 Pascal confocal microscope. Images were processed by ImageJ (US National Institutes of Health) and Adobe Illustrator software (Adobe).

Aggression assays. The fighting chamber was circular, consisting of a central pad of food with a radius of 4 mm and an outer area without food with a radius of 7 mm. The height was 3.5 mm. Newly emerged flies were collected on the first day of eclosion and isolated in a 1.5-ml Eppendorf tube containing 0.5 ml of food. Behavioral tests were carried out at 25 °C and 60% humidity on flies aged 5 to 7 d, except in *shi^{ts}* experiments. *Tdc2*/*UAS-shi^{ts}* flies and controls

were reared and isolated for 10–12 d at 18 °C, followed by aggression tests at either 23 °C (permissive temperature) or 31 °C (restrictive temperature). In our experience, the fighting chambers affected aggression and should thus be carefully prepared; food was melted and delivered into the lid of a 1.5-ml Eppendorf tube. The lid was then put into the chamber and surrounded by 0.8% agarose. On the day of the assay, apple juice with 25% sucrose and 25% yeast was prepared. We added 20 µl of sucrose/yeast solution onto the surface of the food patch in the fighting chamber and let it air dry. Two flies were placed into the fighting chamber and allowed to stay for 30 min before the fighting chambers were used.

Two flies were transferred into a blue pipette tip with the narrow end that was cut to fit the hole of a paper lid that was placed on the fighting chamber. The paper lid was then rapidly replaced with a 20-mm × 20-mm glass cover slip. Six chambers could be recorded by a video camera under a fluorescent lamp; its intensity near the fighting chambers was adjusted to approximately 3,000 lux. Experimental and control groups were taped simultaneously under the same camera. Recordings lasted 10 min after the last two flies were introduced.

In the mutant versus wild-type procedure, mutants and controls were anaesthetized under light CO_2 and marked on the thorax with red or green acrylic paint, respectively. The colors of the mutants and wild-type flies varied in different chambers so that mutants and wild-type flies were equally represented by red or green paints. Painted flies were allowed to recover for 24 h before aggression assays were carried out. The fly that initiated the fighting by directing any aggressive patterns (lunging in most cases) was defined as the initiator during an encounter. Agonistic encounters were defined as those containing at least one lunging, holding or boxing and tussling pattern. An agonistic encounter was terminated when no aggressive patterns were displayed for more than 2 s (ref. 32). In most of the encounters, the initiators, which were also dominant in the fighting, tended to lunge more frequently down on the opponent, trying to push it away from the food pad. Thus, the fly that succeeded in forcing the opponent off was considered to be the winner of that encounter, with the fly being driven off of the food pad being the loser.

Male-female courtship. The male-female courtship assay was carried out in a small, 8-mm (diameter) × 3-mm (height) courtship chamber as described previously[34]. Canton-S virgin females that were aged for 3–5 d served as mating objects. Courtship behavior was videotaped for 30 min after a virgin female and a test male were introduced. We calculated the courtship index.

Male-male courtship. The procedure was similar to the male-female courtship assay, except that two males were introduced.

Mating competition. We used 8-mm × 3-mm courtship chambers for the mating competition assay[44]. Newly eclosed mutants and wild-type flies were collected and isolated in Eppendorf tubes. On the fourth day of eclosion, mutants and controls were anaesthetized and marked with acrylic paint, as described for the aggression assays. On the fifth day, a mutant and a wild-type with different colors were introduced into a courtship chamber containing a Canton-S virgin female and were videotaped for 30 min. We measured the percentage of copulation success for both mutants and controls.

Sexual discrimination. As described previously[45], a decapitated wild-type virgin female and a decapitated wild-type male were placed in the courtship chamber. The test fly was introduced into the chamber and the total time during which it directed courtship behavior toward either the decapitated female or the decapitated male was counted.

Locomotion. Locomotion of individual flies in a small, round chamber were videotaped and analyzed using Ethovision software (Noldus). The average speeds of wild-type and mutants during a period of 4 min were calculated. Similar results were obtained with a line-crossing assay that was carried out as described previously[46].

Odor sensitivity. Flies were deprived of food for 3–6 h in vials and were then exposed to different concentrations of benzaldehyde that was provided from one end of the vials. The avoidance index was calculated as the number of flies that stayed away from the odor source[47].

Drug treatment. We grouped 10 Canton-S flies in vials and aged them for 4 d before drug treatment. CDM (Sigma) was dissolved in a 5% sucrose (wt/vol) solution at the appropriate concentrations. A saturating amount (2 ml) was added into vials that were each lined with 7-cm × 7-cm filter paper (Waterman, 3MM). Flies were then introduced into the vials for 24 h before the behavioral tests were carried out.

Heat shock treatment. For heat-induced expression of TβH, flies, grouped in Eppendorf tubes by pairs, were aged for 4 d. The tubes were then kept at 37 °C in an incubator for 30 min. After the heat shock, flies recovered for 3 h before we carried out behavioral tests.

For the *Gal80^{ts}* experiments, both the experimental and control flies were crossed and cultured at 18 °C to prevent leaky expression of NaChBac occurring at room temperature. Flies were aged in Eppendorf tubes for 9–10 d under 18 °C before heat shock. Flies were cultured at 32 °C for 18 h and subsequently moved to 23 °C for a 2-h recovery. We also moved control flies that were not subjected to heat shock from 18 °C to 23 °C for a period of 2 h. Behavioral tests were performed at 23 °C.

Statistical analysis. For the data that are not normally distributed, we used the Mann Whitney test or the Kruskal-Wallis ANOVA test for analyzing unpaired groups. The Kruskal-Wallis ANOVA test, followed by Dunn's multiple comparison test, was used to identify significant differences between the groups ($P < 0.05$). For paired groups, Wilcoxon signed rank test was applied for nonparametric data. All the statistical analyses were carried out with Prism 4 software (GraphPad).

Note: Supplementary information is available on the Nature Neuroscience website.

ACKNOWLEDGMENTS

We are grateful to M. Zhou and the Antibody Center of the National Institute of Biological Sciences for the antibody to TβH; J. Hirsh, M. Monastirioti, P. Shen, B. White, C.-F. Wu, R. Greenspan, P. Salvaterra, T. Kitamoto and the Bloomington Stock Center for fly stocks; X. Wu for participation in early experiments; Y. Jiang for help with statistics; X. Jia for technical assistance; and the Rao lab members for helpful discussions.

Published online at http://www.nature.com/natureneuroscience/
Reprints and permissions information is available online at http://npg.nature.com/reprintsandpermissions/

1. Darwin, C. *The Descent of Man and Selection in Relation to Sex* (John Murray, London, England, 1871).
2. Lorenz, K.Z. *On Aggression* (Harcourt, Brace and World, New York, 1963).
3. Scott, J.P. Genetic differences in the social behavior of inbred strains of mice. *J. Hered.* **33**, 11–15 (1942).
4. Brunner, H.G., Nelen, M., Breakefield, X.O., Ropers, H.H. & van Oost, B.A. Abnormal behavior associated with a point mutation in the structural gene for monoamine oxidase A. *Science* **262**, 578–580 (1993).
5. Sturtevant, A.H. Experiments on sex recognition and the problem of sexual selection in *Drosophila*. *Anim. Behav.* **5**, 351–366 (1915).
6. Jacobs, M.E. Influence of light on mating of *Drosophila melanogaster*. *Ecology* **41**, 182–188 (1960).
7. Dow, M.A. & von Schilcher, F. Aggression and mating success in *Drosophila melanogaster*. *Nature* **254**, 511–512 (1975).
8. Jacobs, M.E. Influence of β-alanine on mating and territorialism in *Drosophila melanogaster*. *Behav. Genet.* **8**, 487–502 (1978).
9. Hoffmann, A.A. A laboratory study of male territoriality in the sibling species *Drosophila melanogaster* and D. simulans. *Anim. Behav.* **35**, 807–818 (1987).
10. Hoffmann, A.A. Territorial encounters between *Drosophila* males of different sizes. *Anim. Behav.* **35**, 1899–1901 (1987).
11. Hoffmann, A.A. Geographic variation in the territorial success of *Drosophila melanogaster* males. *Behav. Genet.* **19**, 241–255 (1989).
12. Hoffmann, A.A. The influence of age and experience with conspecifics on territorial behaviour in *Drosophila melanogaster*. *J. Insect Behav.* **3**, 1–12 (1990).
13. Hoffmann, A.A. & Cacoyianni, Z. Selection for territoriality in *Drosophila melanogaster*: correlated responses in mating success and other fitness components. *Anim. Behav.* **38**, 23–34 (1989).
14. Hoffmann, A.A. & Cacoyianni, Z. Territoriality in *Drosophila melanogaster* as a conditional strategy. *Anim. Behav.* **40**, 526–537 (1990).

15. Ueda, A. & Kidokoro, Y. Aggressive behaviours of female *Drosophila melanogaster* are influenced by their social experience and food resources. *Physiol. Entomol.* **27**, 21–28 (2002).
16. Nilsen, S.P., Chan, Y.B., Huber, R. & Kravitz, E.A. Gender-selective patterns of aggressive behavior in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **101**, 12342–12347 (2004).
17. Vrontou, E., Nilsen, S.P., Demir, E., Kravitz, E.A. & Dickson, B.J. *fruitless* regulates aggression and dominance in *Drosophila*. *Nat. Neurosci.* **9**, 1469–1471 (2006).
18. Dierick, H.A. & Greenspan, R.J. Molecular analysis of flies selected for aggressive behavior. *Nat. Genet.* **38**, 1023–1031 (2006).
19. Edwards, A.C., Rollmann, S.M., Morgan, T.J. & Mackay, T.F. Quantitative genomics of aggressive behavior in *Drosophila melanogaster*. *PLoS Genet.* **2**, e154 (2006).
20. Dierick, H.A. & Greenspan, R.J. Serotonin and neuropeptide F have opposite modulatory effects on fly aggression. *Nat. Genet.* **39**, 678–682 (2007).
21. Livingstone, M.S., Harris-Warrick, R.M. & Kravitz, E.A. Serotonin and octopamine produce opposite postures in lobsters. *Science* **208**, 76–79 (1980).
22. Edwards, D.H. & Kravitz, E.A. Serotonin, social status and aggression. *Curr. Opin. Neurobiol.* **7**, 812–819 (1997).
23. Stevenson, P.A., Hofmann, H.A., Schoch, K. & Schildberger, K. The fight and flight responses of crickets depleted of biogenic amines. *J. Neurobiol.* **43**, 107–120 (2000).
24. Stevenson, P.A., Dyakonova, V., Rillich, J. & Schildberger, K. Octopamine and experience-dependent modulation of aggression in crickets. *J. Neurosci.* **25**, 1431–1441 (2005).
25. Baier, A., Wittek, B. & Brembs, B. *Drosophila* as a new model organism for the neurobiology of aggression? *J. Exp. Biol.* **205**, 1233–1240 (2002).
26. Certel, S.J., Savella, M.G., Schlegel, D.C. & Kravitz, E.A. Modulation of *Drosophila* male behavioral choice. *Proc. Natl. Acad. Sci. USA* **104**, 4706–4711 (2007).
27. Hoyer, S.C. *et al.* Octopamine in male aggression of *Drosophila*. *Curr. Biol.* **18**, 159–167 (2008).
28. Monastirioti, M. Distinct octopamine cell population residing in the CNS abdominal ganglion controls ovulation in *Drosophila melanogaster*. *Dev. Biol.* **264**, 38–49 (2003).
29. Schwaerzel, M. *et al.* Dopamine and octopamine differentiate between aversive and appetitive olfactory memories in *Drosophila*. *J. Neurosci.* **23**, 10495–10502 (2003).
30. Valzelli, L. Aggressive behavior induced by isolation. in *Aggressive Behavior* (Eds. Garattini, S. and Sigg, E.B.) 70–76 (Wiley, New York, 1969).
31. Wang, L., Heiko Dankert, H., Perona, P. & Anderson, D.J. A common genetic target for environmental and heritable influences on aggressiveness in *Drosophila*. *Proc. Natl. Acad. Sci. USA* **105**, 5657–5663 (2008).
32. Chen, S., Lee, A.Y., Bowens, N.M., Huber, R. & Kravitz, E.A. Fighting fruit flies: a model system for the study of aggression. *Proc. Natl. Acad. Sci. USA* **99**, 5664–5668 (2002).
33. Kitamoto, T. Conditional modification of behavior in *Drosophila* by targeted expression of a temperature-sensitive *shibire* allele in defined neurons. *J. Neurobiol.* **47**, 81–92 (2001).
34. Villella, A. *et al.* Extended reproductive roles of the *fruitless* gene in *Drosophila melanogaster* revealed by behavioral analysis of new *fru* mutants. *Genetics* **147**, 1107–1130 (1997).
35. Luan, H. *et al.* Functional dissection of a neuronal network required for cuticle tanning and wing expansion in *Drosophila*. *J. Neurosci.* **26**, 573–584 (2006).
36. Cole, S.H. *et al.* Two functional, but noncomplementing, *Drosophila* tyrosine decarboxylase genes: distinct roles for neural tyramine and octopamine in female fertility. *J. Biol. Chem.* **280**, 14948–14955 (2005).
37. Kitamoto, T. Conditional disruption of synaptic transmission induces male-male courtship behavior in *Drosophila*. *Proc. Natl. Acad. Sci. USA* **99**, 13232–13237 (2002).
38. Ganguly-Fitzgerald, I., Donlea, J. & Shaw, P.J. Waking experience affects sleep need in *Drosophila*. *Science* **313**, 1775–1781 (2006).
39. Kravitz, E.A. Hormonal control of behavior: amines and the biasing of behavioural output in lobsters. *Science* **241**, 1775–1781 (1988).
40. Adamo, S.A., Linn, C.E. & Hoy, R.R. The role of neurohormonal octopamine during 'fight or flight' behaviour in the field cricket *Gryllus bimaculatus*. *J. Exp. Biol.* **198**, 1691–1700 (1995).
41. Roeder, T. Tyramine and octopamine: ruling behavior and metabolism. *Annu. Rev. Entomol.* **50**, 447–477 (2005).
42. Sallinen, J., Haapalinna, A., Viitamaa, T., Kobilka, B.K. & Scheinin, M. Adrenergic α2C-receptors modulate the acoustic startle reflex, prepulse inhibition and aggression in mice. *J. Neurosci.* **18**, 3035–3042 (1998).
43. Marino, M.D., Bourdelat-Parks, B.N., Cameron Liles, L. & Weinshenker, D. Genetic reduction of noradrenergic function alters social memory and reduces aggression in mice. *Behav. Brain Res.* **161**, 197–203 (2005).
44. Bray, S. & Amrein, H. A putative *Drosophila* pheromone receptor expressed in male-specific taste neurons is required for efficient courtship. *Neuron* **39**, 1019–1029 (2003).
45. Svetec, N. & Ferveur, J.F. Social experience and pheromonal perception can change male-male interactions in *Drosophila melanogaster*. *J. Exp. Biol.* **208**, 891–898 (2005).
46. Kulkarni, S.J. & Hall, J.C. Behavioral and cytogenetic analysis of the cacophony courtship song mutant and interacting genetic variants in *Drosophila melanogaster*. *Genetics* **115**, 461–475 (1987).
47. Anholt, R.R., Lyman, R.F. & Mackay, T.F. Effects of single P-element insertions on olfactory behavior in *Drosophila melanogaster*. *Genetics* **143**, 293–301 (1996).