Biochemical and Biophysical Research Communications 423 (2012) 557–563

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Mutation of *Drosophila* dopamine receptor DopR leads to male–male courtship behavior

Bin Chen [a,b], He Liu [a,b], Jing Ren [a,b], Aike Guo [a,c,*]

[a] Institute of Neuroscience and State Key Laboratory for Neuroscience, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China
[b] Graduate school of Chinese Academy of Sciences, Beijing, China
[c] State Key Laboratory of Brain and Cognitive Science, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China

ARTICLE INFO

Article history:
Received 26 May 2012
Available online 7 June 2012

Keywords:
Drosophila
Dopamine
Dopamine receptor
Male–male courtship behavior
Mutant

ABSTRACT

In *Drosophila*, dopamine plays important roles in many biological processes as a neuromodulator. Previous studies showed that dopamine level could affect fly courtship behaviors. Disturbed dopamine level leads to abnormal courtship behavior in two different ways. Dopamine up-regulation induces male–male courtship behavior, while down-regulation of dopamine level results in increased sexual attractiveness of males towards other male flies. Until now, the identity of the dopamine receptor involved in this abnormal male–male courtship behavior remains unknown. Here we used genetic approaches to investigate the role of dopamine receptors in fly courtship behavior. We found that a dopamine D_1-like receptor, DopR, was involved in fly courtship behavior. DopR mutant male flies display male–male courtship behavior. This behavior is mainly due to the male's increased propensity to court other males. Expression of functional DopR successfully rescued this mutant phenotype. Knock-down of D_2-like receptor D2R and another D_1-like receptor, DAMB, did not induce male–male courtship behavior, indicating the receptor-type specificity of this phenomenon. Our findings provide insight into a possible link between dopamine level disturbance and the induced male–male courtship behavior.

1. Introduction

Courtship behavior is one of the most complex behaviors in *Drosophila*, involving interactions of multiple sensory modalities between males and females. When courting a female, a male fly orients to the female, follows her, vibrates his wings toward her, taps her with forelegs, licks her abdomen, attempts to copulate with her, and finally succeeds in copulation[1–4]. These step-by-step rituals provide a model for the investigation of genes and neural circuits underlying this complex behavior.

The most important genetic pathway that controls courtship behavior of *Drosophila* is the sex determination hierarchy, which consists of four genes, *Sex-lethal* (*Sxl*), *transformer* (*tra*), *fruitless* (*fru*) and *doublesex* (*dsx*) [5,6]. Sexually specific transcription of *tra*, *fru* and *dsx* leads to the anatomical and behavioral differences between males and females, including sexual behaviors [7–10].

Dopamine, via different receptors [11], regulates many aspects of fly development and behaviors, such as arousal [12–14], classical olfactory learning [15–19] and choice behavior [20]. Our previous work showed that dopamine level was important for normal fly courtship behavior and that abnormal dopamine level led to disturbed courtship behavior. When dopamine level was up-regulated, male flies courted wild type males vigorously [21], while males with down-regulated dopamine level displayed increased sexual attractiveness toward wild type males [22]. These results indicate that a medium dopamine level is required for normal male courtship behavior.

Dopamine receptors are categorized into two families, D_1-like and D_2-like family. When activated, D_1-like receptors activate adenylyl cyclase, while D_2-like receptors inhibit it [23]. Several dopamine receptors have been identified in *Drosophila* [24–26]. Although the important role of dopamine level in regulating courtship behavior has been determined, the identity of the receptor through which this regulation is accomplished remains unclear. Here, we used behavior assays together with genetic, electrophysiological and molecular methods to investigate the role of DopR, a D_1-like receptor, in *Drosophila* courtship behavior. Our studies indicate that mutation of DopR can lead to male–male courtship behavior.

2. Materials and methods

2.1. Fly stocks and fly culture

CS flies were used as wild type flies. $DopR^{PL00420}$/TM3,Sb (Bloomington *Drosophila* Stock Center) and $DopR^{f02676}$/TM6B (The

* Corresponding author at: State Key Laboratory of Brain and Cognitive Science, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China.
E-mail address: akguo@ion.ac.cn (A. Guo).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.06.003

Exelixis Collection at the Harvard Medical School) is the DopR mutant lines used in the experiments. *elav*-Gal4 was a pan-neural expressing Gal4 driver. V105324 and V11471 (Vienna *Drosophila* RNAi Center) are DAMB (DAMB is another D_1-like receptor, different from DopR) and D2R (a D_2-like receptor) RNA interference strains under the control of UAS. Flies were raised under 12 h day/night photo cycles, at 25 °C and 60% relative humidity. All flies used for courtship assays were collected within 4 h after eclosion and reared individually in standard unyeasted food vials. For all behavior tests, 5–7 days old flies were used.

2.2. Behavior assays

2.2.1. Paired courtship assay

A courter fly and a decapitated courtee fly were transferred into a courtship chamber without anesthesia. The behavior of the courter towards the courtee was recorded with a camera and analyzed later. Courtship Index (CI) is the percentage of time the courter spent courting the courtee within 5 min [27].

2.2.2. Competitive courtship assay

Two decapitated courtee, one male and one female, were presented to one courter male simultaneously [27]. Preference Index (PI) was calculated according to the following equation: $PI = (T_f - T_m)/300$, where T_f and T_m represent the time courter spent courting the female and the male in 5 min, respectively.

2.2.3. Locomotor activity test

A fly was transferred into a small chamber, which was divided into two identical parts by a midline. The number of times that a fly crossed the midline within 5 min was used to measure the fly's locomotor activity [27].

2.2.4. Olfactory sensitivity test

Olfactory sensitivity test was used to test fly's avoidance of 4-methylcyclohexanol (MCH) and 3-octanol (OCT) at different concentrations as previously described [28].

2.2.5. Proboscis extension response

Flies were fixed on slides after overnight starvation. After recovery for 2 h in a humid box, proboscis extension response was elicited by touching tarsi of fly's forelegs with a drop of sucrose solution. Five trials were performed for each fly, and the number of proboscis extension was recorded. To avoid adaptation, the time interval between trials was at least 30 s [28].

2.2.6. Two-choice assay

Two-choice assay was used to test fly's gustatory sensitivity for bitter material [29]. After a 18 h starvation period, 50 flies were transferred into a 60-well plate containing alternating wells of 1 mM sucrose (containing 0.25 mg/ml indigo carmine, 1% agar) and 5 mM sucrose plus 20 mM caffeine (containing 0.5 mg/ml sulforhodamine, 1% agar). Flies were fed for 4 h in the dark at 25 °C. According to the color of flies abdomen (red, blue or purple), flies were categorized and scored after the plate was frozen at −20 °C. The Preference Index (PI) was calculated according to the following equation: $PI = (N_{red} + 0.5\ N_{purple})/(N_{red} + N_{blue} + N_{purple})$, where N_{red}, N_{blue} and N_{purple} represent the number of flies with red, blue and purple abdomens.

2.3. Electroretinogram recordings (ERG)

We modified the protocol described previously [30]. A single fly was anesthetized with ice and immobilized in dental wax. The reference microelectrode was filled with 3 M KCl and gently inserted into the thorax. The recording microelectrode was filled with 3 M KCl and inserted into the center of the eye. Fly was dark adapted for 1 min. A 1s light pulse was presented every 10 s for five times. ERG signal was recorded with Multiclamp 700B (Axon CNS), and analyzed with the software Clampfit 10.2.

2.4. Molecular biology

2.4.1. RNA isolation

Fly heads were collected after 200 flies were snap frozen with liquid nitrogen. RNAs were extracted following standard Trizol method.

2.4.2. Real-time quantitative PCR (qPCR)

RNA quality was assessed using the Lab-on-a-Chip 2100 Bioanalyzer (Agilent) platform. 2 mg total RNA was treated with RQ1 DNase (Promega), and then reverse-transcribed with oligo(dT) primers and Superscript III reverse transcriptases (Invitrogen). Real-time PCR was carried out with SYBR Premix Ex Taq™ II kit (Takara) using an ABI PRISM 7000 real-time PCR Detection system (Applied Biosystems). The relative mRNA level was calculated using the comparative C_T method [31]. *rp49* was used as the reference gene. Three repeats were performed for each sample, and data from three independent samples were collected and analyzed. Primer sequences used for qPCR are as follows: *rp49*: 5′-CCAAGGA CTTCATCCGCCACC-3′, 5′-GCGGGTGCGCTTGTTCGATCC-3′; *DopR*: 5′-GAAGTCCATCAAGGCGGTAA-3′, 5′-AGCCAGGTGAGGATCTTGAA-3′; DAMB(*DopR2*): 5′-CGCGAATTCCTGCGAGGGATGG CGAGATG-3′, 5′-CGCTCTAGAGTGCGAATGGGAGTGCGAGTG-3′; *D2R*: 5′-CAC AAG GCCTCGAAAAAGAA-3′, 5′-GCGAAACTCGGGATTGAATA-3′ [32].

3. Results and discussion

3.1. DopR mutation induced male–male courtship behavior

Since up-regulation of dopamine level induces male–male courtship, we tried to identify the dopamine receptor involved in this male–male courtship behavior. We focused on a D_1-like receptor, DopR, which is localized mainly in the mushroom bodies, and the central complex [33]. When paired courtship assays were performed, one homozygous DopR mutant line, $DopR^{PL00420}$ [14], showed strong male–male courtship (Courtship Index (CI) = 38.4 ± 4.4, $N = 29$) (Supplementary Movie 1 and Fig. 1A). Unlike dopamine level up-regulated flies and some other mutant flies, when several $DopR^{PL00420}$ mutant male flies were transferred into a small chamber, no courtship chaining was observed [1,2]. The male–male courtship induced by DopR mutation could be the result of two different effects, an active courtship towards the target by $DopR^{PL00420}$ mutant male, or increased sexual attractiveness of $DopR^{PL00420}$ male to the courter. Paired courtship assay with one mutant male and one wild type male was used to investigate which effect results in this male–male courtship behavior. Heterozygous $DopR^{PL00420}$ ($DopR^{PL00420}$/+) mutants show nearly no courtship towards wild type male (CI = 3.4 ± 2.6, $N = 24$). However, homozygous male $DopR^{PL00420}$ mutants court wild type male (CI = 17.0 ± 3.5, $N = 35$) (Supplementary movie 2). Real-time quantitative PCR (qPCR) showed that $DopR^{PL00420}$/$DopR^{PL00420}$ male flies showed lower DopR mRNA level compared to wild type males (75% of wild type DopR mRNA level) (Fig. 1D). For some unknown reasons, no courtship was observed when another homozygous DopR mutant line, $DopR^{f02676}$ [14], was tested (CI = 1.2 ± 0.6, $N = 21$). Interestingly, although both heterozygous and homozygous $DopR^{f02676}$ mutant display no male–male courtship behavior, the trans-heterozygous mutant male $DopR^{PL00420}$/$DopR^{f02676}$ shows courtship towards wild type male fly (CI = 11.7 ± 3) (Fig. 1B) and this male–male courtship behavior is statistically greater than

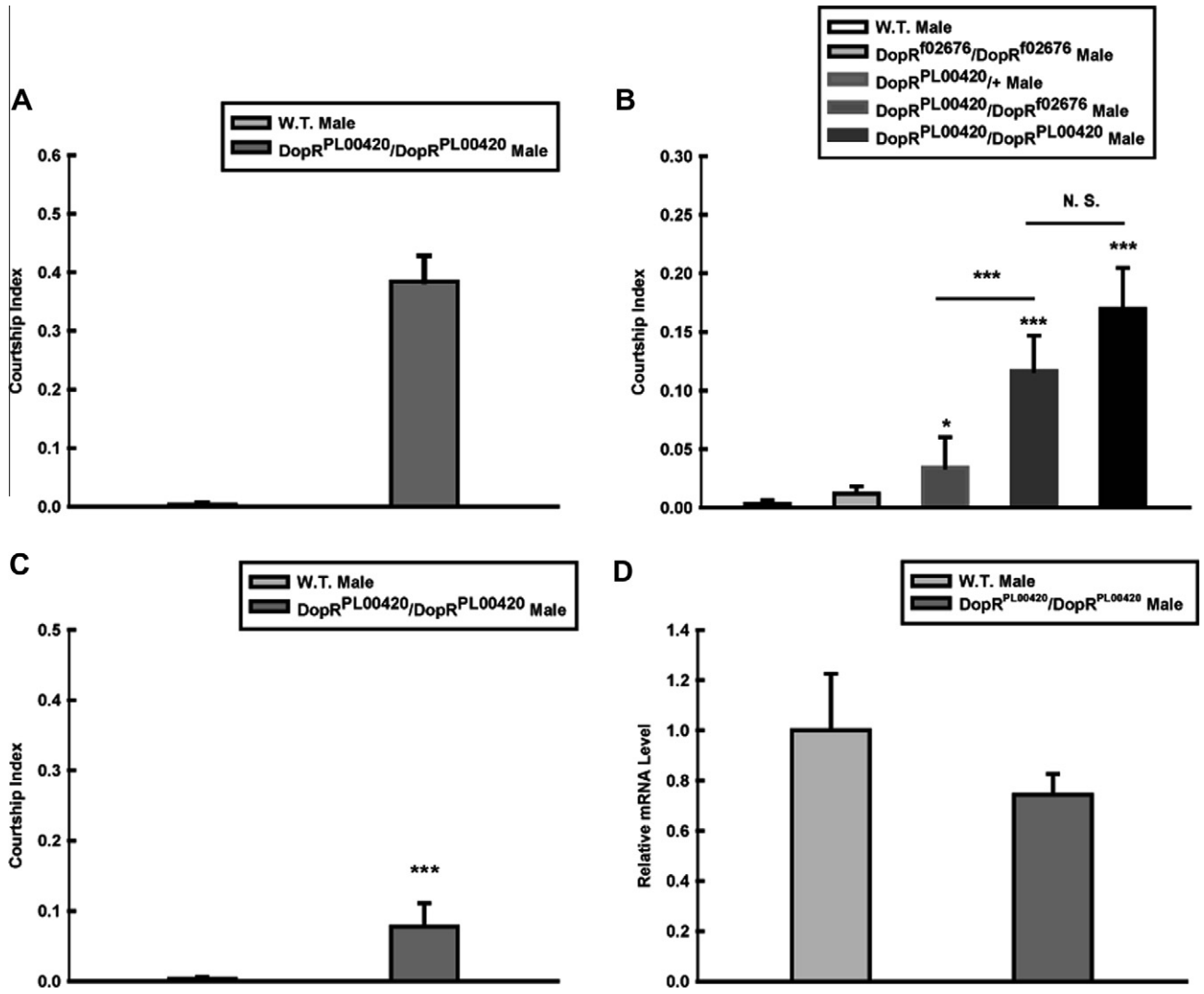

Fig. 1. DopR mutant male fly displays male–male courtship behavior. (A) Courtship Index (CI) for homozygous DopR^{PL00420} mutant male towards DopR^{PL00420} mutant target male ($N = 29$) and CI for wild type male towards wild type male ($N = 22$). (B) CI for wild type male and different DopR mutant males towards wild type males ($N = 22$, 21, 24, 23 and 35). (C) CI for wild type male towards homozygous DopR^{PL00420} mutant male ($N = 23$). (D) Homozygous DopR^{PL00420} mutant male DopR mRNA level was lower than that of wild type male. Values are shown as means ± SEM. Mann–Whitney Rank Sum Test was used to evaluate the statistical significances of differences. ∗, ∗∗∗, *N. S.* stands for $P < 0.05$, $P < 0.001$ and no significant difference, respectively.

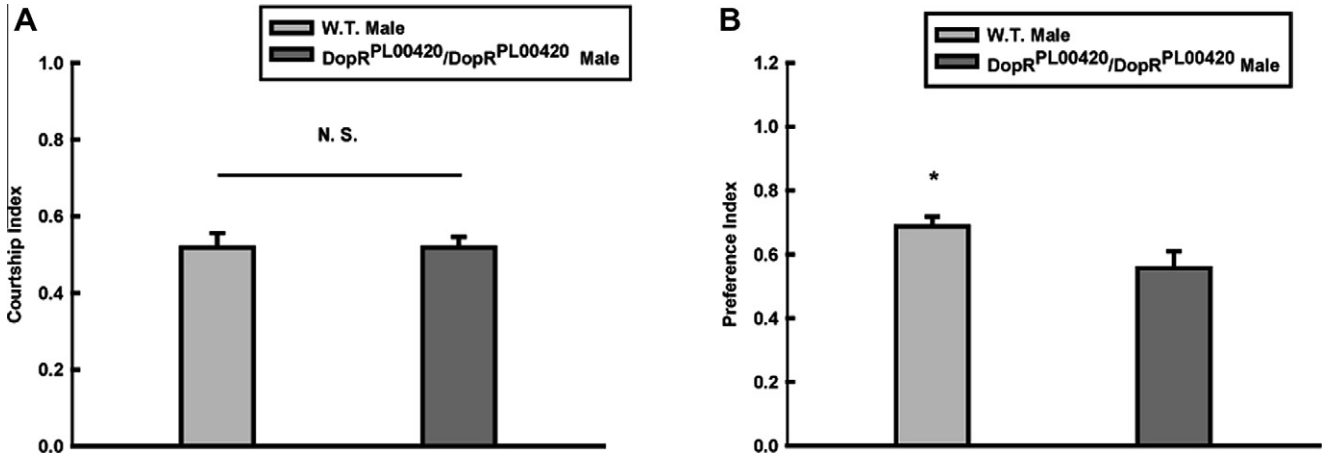

Fig. 2. DopR mutant shows normal sexual appeal and female preference compared to wild type male. A. Courtship Index (CI) for wild type male ($N = 29$) and homozygous DopR^{PL00420} mutant male ($N = 37$) towards wild type female. B. Preference Index (PI) for wild type male ($N = 21$) and homozygous DopR^{PL00420} mutant male ($N = 19$) in competitive courtship assay. Values are shown as means ± SEM. Student's *t*-test was used to evaluate the statistical significances of differences. ∗ and *N. S.* stands for $P < 0.05$ and no significant difference, respectively.

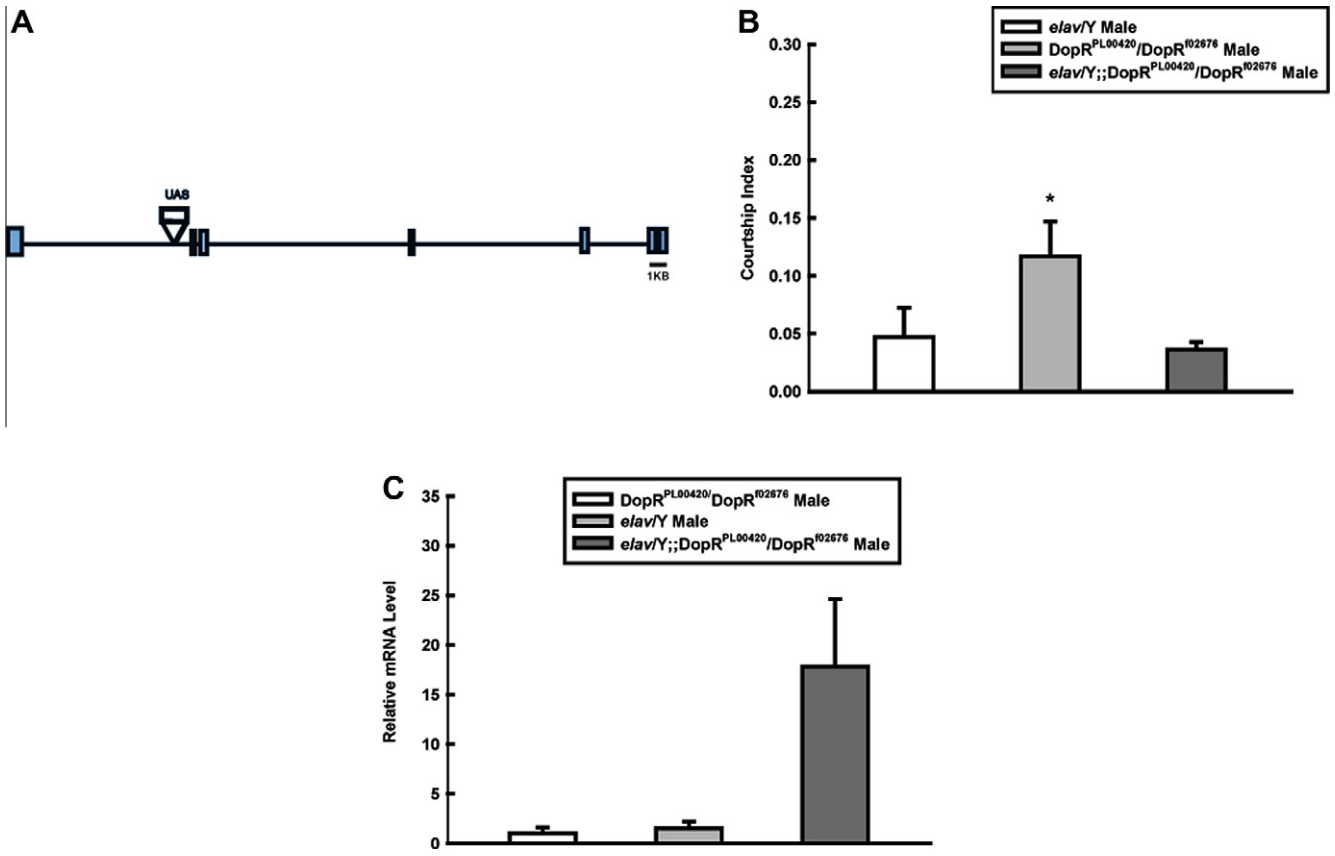

Fig. 3. Rescue of male–male courtship phenotype by expression of DopR. (A) Structure of the DopR^{f02676} insertional allele, the UAS is in the first intron. (B) Expression of functional DopR rescues the male–male courtship phenotype. Courtship Index (CI) for *elav*/Y; DopR^{PL00420}/DopR^{f02676} ($N = 33$), DopR^{PL00420}/DopR^{f02676} ($N = 23$), and *elav*/Y male flies ($N = 20$). (C) *elav*/Y; DopR^{PL00420}/DopR^{f02676} showed higher DopR mRNA level compared to DopR^{PL00420}/DopR^{f02676} and *elav*/Y flies. Values are shown as means ± SEM. Mann–Whitney Rank Sum Test was used to evaluate the statistical significances of differences. $*$ stands for $P < 0.05$.

the level displayed by heterozygous DopR^{PL00420}/+ mutants (CI = 11.7 ± 3 vs. CI = 3.4 ± 2.6). Furthermore, courtship behavior of wild type males was only slightly (but statistically significantly) increased (CI = 7.8 ± 3.3, $N = 23$) (Fig. 1C). Therefore, we focused on the male–male courtship behavior DopR^{PL00420} mutant displayed towards target flies in our following experiments. It is noticeable that the CI for DopR^{PL00420}/ DopR^{PL00420} male towards DopR^{PL00420}/DopR^{PL00420} male is higher than that for DopR^{PL00420}/ DopR^{PL00420} male towards wild type male. A possible reason is that the former CI is a combinatory result of the mutant courter's courtship behavior towards target and the slightly increased attractiveness of the mutant courtee. Our results suggest that DopR mutation leads to male–male courtship behavior mainly through the increased propensity of mutant males to court other males.

3.2. The male–male courtship displayed by DopR mutant fly is not caused by an increased sexual appeal

The male–male courtship displayed by DopR mutant could be caused by an increased sexual appeal. To examine whether it is the case for our observations, we tested flies sexual appeal towards wild type female. No difference was observed between DopR mutant males (CI = 51.9 ± 2.8, $N = 36$) and wild type males (CI = 51.9 ± 3.7, $N = 29$) (Fig. 2A), indicating that the male–male courtship is not due to increased sexual appeal.

3.3. Male–male courtship behavior displayed by DopR mutant is not due to incapability of distinguishing between female and male targets

Previous reports indicate that male–male courtship could be induced by flies inability to distinguish female from male targets [34]. We performed competitive courtship assay [27] to test whether the DopR mutant is capable of distinguishing between female and male. Preference index (PI) was used as a measurement for male's courtship preference. A positive PI demonstrates that the courter prefers female, and a negative PI stands for male-preference. If the courter displays no preference, PI would be zero, indicating that the courter can't distinguish between male and female. The assay results showed that DopR^{PL00420} mutant male displayed nearly exclusive courtship towards the female (PI = 55.7 ± 5.3, $N = 19$) (Fig. 2B). Therefore the male–male courtship induced by DopR mutation is not due to gender blindness.

3.4. DopR mutant flies showed normal sensory and locomotor activity

Locomotor and sensory defects can also affect normal courtship behavior of *Drosophila*. To examine whether DopR mutants display any of these defects, locomotor activity, general olfactory and gustatory sensitivity tests were performed [27,28]. Locomotor activity tests showed that DopR mutants display normal locomotor activity as control flies (Supplementary Fig. 1A). The mutants are also as sensitive as wild type flies to different concentrations of aversive odors (4-methylcyclohexanol and 3-octanol, MCH and OCT) in olfactory sensitivity tests (Supplementary Figs. 1B and 1C). The mutants are even more sensitive towards OCT. Proboscis extension response tests [28] showed that DopR mutants and wild type flies are equally sensitive towards sweet material (different concentrations of sucrose) (Supplementary Fig. 2B). Two-choice assays [29] results suggested that the mutant flies are as sensitive as wild type towards bitter material (caffeine) (Supplementary Figs. 2A and 2C). Therefore the male–male courtship displayed by DopR mutant flies

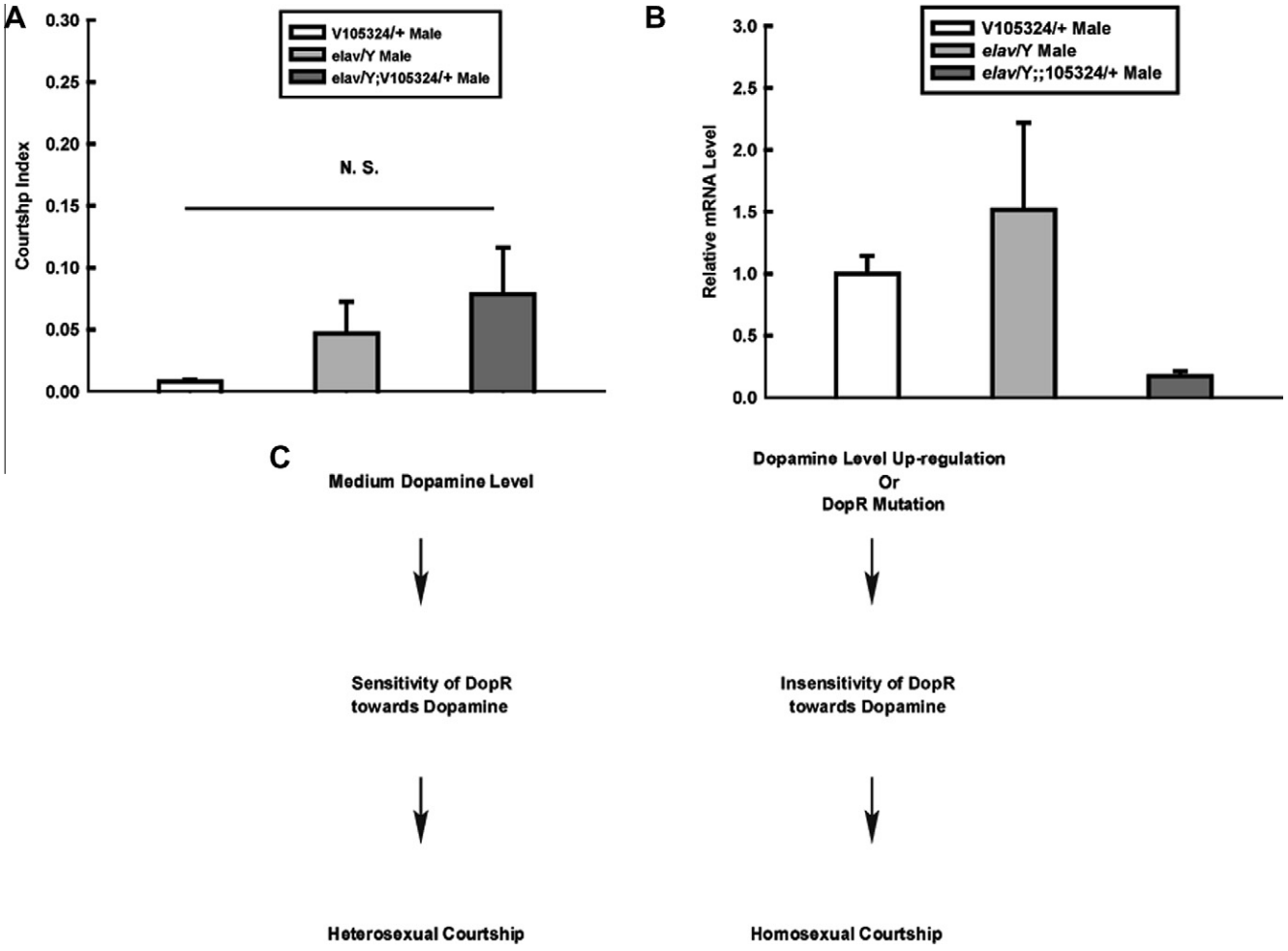

Fig. 4. Knock-down of DAMB doesn't induce male–male courtship behavior. (A) Courtship Index (CI) for fly with pan-neural knock-down of DAMB ($N = 19$) and its controls (V105324/+ $N = 15$, *elav*/Y $N = 20$). (B) DAMB mRNA level was knocked down in *elav*/Y; V105324/+ flies. (C) A hypothetical mechanism of DopR insensitivity for male–male courtship behavior induced by DopR mutation. Values are shown as means ± SEM. Mann–Whitney Rank Sum Test was used to evaluate the statistical significances of differences. $**$, *N. S.* stands for $P < 0.01$, no significant difference, respectively.

is unlikely to be caused by abnormal locomotor activity, or general sensory defects.

Visual signal is also an important sensory input for courtship behavior [2]. We recorded electroretinograms (ERGs) [30] to test whether the DopR mutant displays vision defects. The results show that $DopR^{PL00420}$ mutant flies display normal ERGs ($DopR^{PL00420}$/$DopR^{PL00420}$ ERG amplitude = 12.1 ± 0.72, $N = 10$, Wild type ERG amplitude = 12.9 ± 0.98, $N = 9$) (Supplementary Fig. 2D), indicating that the male–male courtship is unlikely to be caused by abnormal photoreceptor function.

3.5. Rescue of the male–male courtship phenotype by expression of functional DopR

To further confirm our observation that DopR mutation leads to male–male courtship, we performed a DopR rescue experiment. The $DopR^{f02676}$ mutation was caused by a piggyBac transposon insertion into the first intron of the DopR allele. The UAS element in the transposon is functional as a transcription activating sequence under the control of Gal4 protein [14] (Fig. 3A). Transcription from the $DopR^{f02676}$ mutant gene is predicted to produce a truncated protein(s) with a short-ended extracellular domain. Previous studies reported that the missing N-terminal was nonessential for DopR function [35]. Therefore spatially specific rescue can be accomplished in flies with a Gal4 transgene and a $DopR^{f02676}$ mutant allele. This strategy has been successfully used in previous studies [14,17]. Paired courtship assays showed that *elav*/Y; $DopR^{PL00420}$/$DopR^{f02676}$ males displayed reduced male–male courtship behavior (CI = 3.6 ± 0.65, $N = 33$) compared to trans-heterozygous $DopR^{PL00420}$/$DopR^{f02676}$ males (Fig. 3B). Real-time quantitative PCR tests showed that DopR mRNA level was much higher in *elav*/Y;; $DopR^{PL00420}$/$DopR^{f02676}$ (17.8 folds of DopR mRNA level compared to $DopR^{PL00420}$/$DopR^{f02676}$ trans-heterozygous mutant) than $DopR^{PL00420}$/$DopR^{f02676}$ trans-heterozygous mutant and *elav*/Y flies, indicating that the rescue strategy was successful (Fig. 3C). Altogether, these results confirmed our observation that mutant DopR flies displayed male–male courtship behavior, and this homosexual behavior was not likely to be caused by a insertion effect.

3.6. The induction of male–male courtship shows receptor-type specificity

Similar to other animals, dopamine accomplishes its various functions through two types of receptors in *Drosophila*, D_1-like and D_2-like receptors. DAMB, another D_1-like dopamine receptor, has been shown in previous studies [25] to play an important role in classical olfactory learning. To investigate whether the male–male courtship we observed is exclusive to DopR defect, we took advantage of the Gal4/UAS binary system [36] and RNA interference [37] to manipulate DAMB expression level. No obvious

male–male courtship was observed when DAMB was pan-neurally knocked down by driving V105324 with *elav*-Gal4 (Fig. 4A). Real-time quantitative PCR confirmed the knock-down of DAMB (16% of DAMB mRNA level compared to V105324/+) (Fig. 4B). Previous studies have also identified one D_2-like dopamine receptor, D2R [26]. Pan-neural knock-down of D2R did not induce male–male courtship, either (Supplementary Fig. 3A). Results of real-time PCR experiments showed that D2R mRNA was successfully knocked down when V11471 was driven by *elav*-Gal4 (37% of D2R level compared to control) (Supplementary Fig. 3B). These results showed that dopaminergic regulation of courtship behavior is receptor subtype specific, and highlighted the role of DopR in this process.

In *Drosophila*, dopamine has been extensively studied for its roles in various biological processes. Our recent studies indicate that dopamine level is critical for normal courtship behavior. Abnormal dopamine level leads to two different male–male courtship behaviors [21,22].

Our research here shows that at least one D_1-like dopamine receptor, DopR, is involved in courtship behavior regulation. DopR mutant flies show male–male courtship similar to dopamine up-regulated flies. This result provides a possible link between dopamine level disturbance and the male–male courtship it endowed. A recent report suggests that altered neurotransmitter concentration in synaptic cleft could induce male–male courtship behavior, possibly as a result of changed sensitivity of postsynaptic receptors towards the neurotransmitter [34]. It is possible that our results on male–male courtship behavior induced by dopamine level disturbance and DopR mutation were caused by a similar mechanism. Medium concentration of dopamine and postsynaptic DopR level might be necessary for normal courtship behavior. Up-regulated dopamine level may lead to desensitization of DopR to dopamine, which results in DopR being less sensitive to changing dopamine level in the synaptic cleft. We speculate that as a result of this insensitivity to dopamine modulation, male flies display active male–male courtship behavior. Similarly, in DopR mutant flies, dopamine is unable to modulate synaptic connection via DopR, which represents an extreme case for DopR's insensitivity to dopamine, leading to male–male courtship behavior (Fig. 4C).

More than one dopamine receptors have been identified previously in *Drosophila*. Our results showed that not all dopamine receptors are involved in fly courtship behavior. Another D_1-like receptor, DAMB, doesn't seem to be a key factor in *Drosophila* courtship behavior, because knock-down of DAMB did not induce male–male courtship behavior. Similarly, knock-down of D2R, a D_2-like receptor, did not induce male–male courtship behavior. These results indicate that different dopamine receptors might modulate synaptic transmissions underlying different behaviors.

Altogether, our study showed that a D_1-like dopamine receptor, DopR, is involved in *Drosophila* courtship behavior. DopR mutation leads to male–male courtship behavior, and rescue of DopR expression eliminates this homosexual courtship behavior. The male–male courtship behavior was specific to mutants of DopR receptor. and was not seen in flies with deficits in DAMB and D2R receptors. This male–male courtship behavior was similar to that induced by the up-regulated dopamine level, and both might be a result of DopR's insensitivity to dopamine. Further research is required to test this hypothesis and to reveal the mechanism underlying the male–male courtship behavior induced by DopR mutation.

Acknowledgments

This work was supported by the China 973 Program (2011 CBA00400), the National Science Foundation of China program (30921064, 90820008 and 31130027) to A.G.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.06.003.

References

[1] J.C. Hall, The mating of a fly, Science 264 (1994) 1702–1714.
[2] A. Villella, J.C. Hall, Neurogenetics of courtship and mating in *Drosophila*, Adv. Genet. 62 (2008) 67–184.
[3] R.J. Greenspan, J.F. Ferveur, Courtship in *Drosophila*, Annu. Rev. Genet. 34 (2000) 205–232.
[4] D. Yamamoto, J.M. Jallon, A. Komatsu, Genetic dissection of sexual behavior in *Drosophila melanogaster*, Annu. Rev. Entomol. 42 (1997) 551–585.
[5] K.C. Burtis, The regulation of sex determination and sexually dimorphic differentiation in *Drosophila*, Curr. Opin. Cell Biol. 5 (1993) 1006–1014.
[6] T.W. Cline, B.J. Meyer, Vive la difference. males vs. females in flies vs. worms, Annu. Rev. Genet. 30 (1996) 637–702.
[7] E. Demir, B.J. Dickson, Fruitless splicing specifies male courtship behavior in *Drosophila*, Cell 121 (2005) 785–794.
[8] E.J. Rideout, J.C. Billeter, S.F. Goodwin, The sex-determination genes fruitless and doublesex specify a neural substrate required for courtship song, Curr. Biol. 17 (2007) 1473–1478.
[9] A. Villella, J.C. Hall, Courtship anomalies caused by doublesex mutations in *Drosophila melanogaster*, Genetics 143 (1996) 331–344.
[10] K. Kimura, T. Hachiya, M. Koganezawa, T. Tazawa, D. Yamamoto, Fruitless and doublesex coordinate to generate male-specific neurons that can initiate courtship, Neuron 59 (2008) 759–769.
[11] T. Riemensperger, G. Isabel, H. Coulom, K. Neuser, L. Seugnet, K. Kume, M. Iche-Torres, M. Cassar, R. Strauss, T. Preat, J. Hirsh, S. Birman, Behavioral consequences of dopamine deficiency in the *Drosophila* central nervous system, Proc. Natl. Acad. Sci. USA 108 (2011) 834–839.
[12] R. Andretic, B. van Swinderen, R.J. Greenspan, Dopaminergic modulation of arousal in *Drosophila*, Curr. Biol. 15 (2005) 1165–1175.
[13] K. Kume, S. Kume, S.K. Park, J. Hirsh, F.R. Jackson, Dopamine is a regulator of arousal in the fruit fly, J. Neurosci. 25 (2005) 7377–7384.
[14] T. Lebestky, J.S. Chang, H. Dankert, L. Zelnik, Y.C. Kim, K.A. Han, F.W. Wolf, P. Perona, D.J. Anderson, Two different forms of arousal in *Drosophila* are oppositely regulated by the dopamine D1 receptor ortholog DopR via distinct neural circuits, Neuron 64 (2009) 522–536.
[15] B.L. Tempel, M.S. Livingstone, W.G. Quinn, Mutations in the dopa decarboxylase gene affect learning in *Drosophila*, Proc. Natl. Acad. Sci. USA 81 (1984) 3577–3581.
[16] M. Schwaerzel, M. Monastirioti, H. Scholz, F. Friggi-Grelin, S. Birman, M. Heisenberg, Dopamine and octopamine differentiate between aversive and appetitive olfactory memories in *Drosophila*, J. Neurosci. 23 (2003) 10495–10502.
[17] Y.C. Kim, H.G. Lee, K.A. Han, D1 dopamine receptor dDA1 is required in the mushroom body neurons for aversive and appetitive learning in *Drosophila*, J. Neurosci. 27 (2007) 7640–7647.
[18] M.J. Krashes, S. DasGupta, A. Vreede, B. White, J.D. Armstrong, S. Waddell, A neural circuit mechanism integrating motivational state with memory expression in *Drosophila*, Cell 139 (2009) 416–427.
[19] Y. Aso, I. Siwanowicz, L. Bracker, K. Ito, T. Kitamoto, H. Tanimoto, Specific dopaminergic neurons for the formation of labile aversive memory, Curr. Biol. 20 (2010) 1445–1451.
[20] K. Zhang, J.Z. Guo, Y. Peng, W. Xi, A. Guo, Dopamine-mushroom body circuit regulates saliency-based decision-making in *Drosophila*, Science 316 (2007) 1901–1904.
[21] T. Liu, L. Dartevelle, C. Yuan, H. Wei, Y. Wang, J.F. Ferveur, A. Guo, Increased dopamine level enhances male–male courtship in *Drosophila*, J. Neurosci. 28 (2008) 5539–5546.
[22] T. Liu, L. Dartevelle, C. Yuan, H. Wei, Y. Wang, J.F. Ferveur, A. Guo, Reduction of dopamine level enhances the attractiveness of male *Drosophila* to other males, PLoS One 4 (2009) e4574.
[23] B.F. O'Dowd, Structures of dopamine receptors, J. Neurochem. 60 (1993) 804–816.
[24] F. Gotzes, S. Balfanz, A. Baumann, Primary structure and functional characterization of a *Drosophila* dopamine receptor with high homology to human D1/5 receptors, Receptors Channels 2 (1994) 131–141.
[25] K.A. Han, N.S. Millar, M.S. Grotewiel, R.L. Davis, DAMB, a novel dopamine receptor expressed specifically in *Drosophila* mushroom bodies, Neuron 16 (1996) 1127–1135.
[26] M.G. Hearn, Y. Ren, E.W. McBride, I. Reveillaud, M. Beinborn, A.S. Kopin, A *Drosophila* dopamine 2-like receptor: molecular characterization and identification of multiple alternatively spliced variants, Proc. Natl. Acad. Sci. USA 99 (2002) 14554–14559.
[27] A. Villella, D.A. Gailey, B. Berwald, S. Ohshima, P.T. Barnes, J.C. Hall, Extended reproductive roles of the fruitless gene in *Drosophila melanogaster* revealed by behavioral analysis of new fru mutants, Genetics 147 (1997) 1107–1130.
[28] R.R. Anholt, R.F. Lyman, T.F. Mackay, Effects of single P-element insertions on olfactory behavior in *Drosophila melanogaster*, Genetics 143 (1996) 293–301.
[29] L.A. Weiss, A. Dahanukar, J.Y. Kwon, D. Banerjee, J.R. Carlson, The molecular and cellular basis of bitter taste in *Drosophila*, Neuron 69 (2011) 258–272.

[30] F.D. Huang, H.J. Matthies, S.D. Speese, M.A. Smith, K. Broadie, Rolling blackout, a newly identified PIP2-DAG pathway lipase required for *Drosophila* phototransduction, Nat. Neurosci. 7 (2004) 1070–1078.

[31] M.W. Pfaffl, A new mathematical model for relative quantification in real-time RT-PCR, Nucleic Acids Res. 29 (2001) e45.

[32] H.K. Inagaki, S. Ben Tabou de Leon, A.M. Wong, S. Jagadish, H. Ishimoto, G. Barnea, T. Kitamoto, R. Axel, D.J. Anderson, Visualizing neuromodulation in vivo: TANGO-mapping of dopamine signaling reveals appetite control of sugar sensing, Cell 148 (2012) 583–595.

[33] Y.C. Kim, H.G. Lee, C.S. Seong, K.A. Han, Expression of a D1 dopamine receptor dDA1/DmDOP1 in the central nervous system of *Drosophila melanogaster*, Gene Expr. Patterns 3 (2003) 237–245.

[34] Y. Grosjean, M. Grillet, H. Augustin, J.F. Ferveur, D.E. Featherstone, A glial amino-acid transporter controls synapse strength and courtship in *Drosophila*, Nat. Neurosci. 11 (2008) 54–61.

[35] F. Gotzes, A. Baumann, Functional properties of *Drosophila* dopamine D1-receptors are not altered by the size of the N-terminus, Biochem. Biophys. Res. Commun. 222 (1996) 121–126.

[36] A.H. Brand, N. Perrimon, Targeted gene expression as a means of altering cell fates and generating dominant phenotypes, Development 118 (1993) 401–415.

[37] G. Dietzl, D. Chen, F. Schnorrer, K.C. Su, Y. Barinova, M. Fellner, B. Gasser, K. Kinsey, S. Oppel, S. Scheiblauer, A. Couto, V. Marra, K. Keleman, B.J. Dickson, A genome-wide transgenic RNAi library for conditional gene inactivation in *Drosophila*, Nature 448 (2007) 151–156.