Published in final edited form as:
Curr Biol. 2014 March 31; 24(7): 725–730. doi:10.1016/j.cub.2013.12.051.

Sexually Dimorphic Octopaminergic Neurons Modulate Female Postmating Behaviors in *Drosophila*

Carolina Rezával[1], **Tetsuya Nojima**[1], **Megan C. Neville**[1], **Andrew C. Lin**[2], **Stephen F. Goodwin**[1]

[1]Department of Physiology, Anatomy and Genetics, University of Oxford, Sherrington Building, Parks Road, Oxford OX1 3PT, UK

[2]Centre for Neural Circuits and Behavior, University of Oxford, Tinsley Building, Mansfield Road, Oxford OX1 3SR, UK

Summary

Mating elicits profound behavioral and physiological changes in many species that are crucial for reproductive success. After copulation, *Drosophila melanogaster* females reduce their sexual receptivity and increase egg laying [1, 2]. Transfer of male sex peptide (SP) during copulation mediates these postmating responses [1, 3–6] via SP sensory neurons in the uterus defined by coexpression of the proprioceptive neuronal marker *pickpocket* (*ppk*) and the sex-determination genes *doublesex* (*dsx*) and *fruitless* (*fru*) [7–9]. Although neurons expressing *dsx* downstream of SP signalling have been shown to regulate postmating behaviors [9], how the female nervous system coordinates the change from pre- to postcopulatory states is unknown. Here, we show a role of the neuromodulator octopamine (OA) in the female postmating response. Lack of OA disrupts postmating responses in mated females, while increase of OA induces postmating responses in virgin females. Using a novel dsx^{FLP} allele, we uncovered *dsx* neuronal elements associated with OA signaling involved in modulation of postmating responses. We identified a small subset of sexually dimorphic OA/dsx^{+} neurons (approximately nine cells in females) in the abdominal ganglion. Our results are consistent with a model whereby OA neuronal signaling increases after copulation, which in turn modulates changes in female behavior and physiology in response to reproductive state.

Results and Discussion

Lack of octopamine increases female receptivity and disrupts postmating responses

Octopamine (OA) regulates female reproductive physiology in *Drosophila melanogaster*. OA signaling is required for sperm release from storage [10], ovulation, and egg deposition [11–13], and octopaminergic (OA^{+}) neurons in the abdominal ganglion (Abg) help trigger ovulation by modulating muscle contraction in the ovaries and uterus [13–15]. After mating,

Correspondence to: Carolina Rezával; Stephen F. Goodwin.

Correspondence can be addressed to: C.R (carolina.rezaval@dpag.ox.ac.uk) and S.F.G. (stephen.goodwin@dpag.ox.ac.uk).

Accession Numbers

The NCBI GenBank accession number for the DmFLP reported in this paper is KJ507829.

Europe PMC Funders Author Manuscripts

wild-type Drosophila females become temporarily sexually unreceptive to further copulatory attempts, exhibiting rejection behaviors toward courting males such as ovipositor extrusion, and instead focusing mainly on feeding and egg production [1, 2]. We asked whether OA is also required for female postmating behaviors, focusing on two in particular, decreased receptivity and increased egg-laying.

We first evaluated the effects of depleting OA on female behavior by testing a null mutation in the gene that encodes Tyramine β–hydroxylase ($T\beta h^{nM18}$), an enzyme that catalyzes the last step in OA biosynthesis (Figure 1A; [11, 12]). Virgin $T\beta h^{nM18}$ mutant females showed increased receptivity compared to wild-type virgin females; showing a mating latency of less than 3 minutes, compared to ~12 minutes in wild-type females (Figure 1B). As previously reported, mated $T\beta h^{nM18}$ mutant females laid very few eggs (Figure 1C; [12, 13]). In addition, $T\beta h^{nM18}$ mutant females remained highly receptive after copulation (~20% remated, vs. <5% of mated controls; Figure 1D), displaying low levels of ovipositor extrusion when compared with mated controls (Figure 1E). Male courtship index is a measure of the attractiveness of a female: naive males court virgin females persistently but display lower levels of courtship when paired with unreceptive mated females [16]. We found that wild-type males courted $T\beta h^{nM18}$ mated females significantly more than wild-type mated females (>72% vs. >22%; Figure 1F). This difference may stem from altered female behavior, pheromone production, or both. Interestingly, heterozygous $T\beta h^{nM18}/+$ females also showed increased receptivity compared to controls, but not to as great an extent as homozygous $T\beta h^{nM18}$ flies (Figure 1B, D-F), suggesting a dose-dependent effect of OA synthesis.

As Tβh converts tyramine to OA (Figure 1A), $T\beta h^{nM18}$ mutants have elevated tyramine levels [12]. If the changes in receptivity and postmating responses were due to excessive levels of tyramine rather than lack of octopamine, the opposite behavioral effects should be seen in *Tyrosine decarboxylase 2* ($Tdc2^{RO54}$) mutants, which lack both tyramine and OA [11]. However, $Tdc2^{RO54}$ flies phenocopied $T\beta h^{nM18}$ mutants in all assays (Figure 1B-F), supporting our conclusion that behavioral phenotypes seen in $T\beta h^{nM18}$ mutant females are mainly due to a lack of OA in the nervous system.

Increasing octopamine levels reduces receptivity and triggers postmating responses in wild-type females and *Tβh* mutant females

If depletion of OA in females disrupts postmating responses, then elevation of OA levels in virgin females may induce postmating responses. To test this possibility, we fed 1-day-old virgin females on OA-containing food for 6 days [12], and then assessed them for receptivity. Indeed, for both wild-type and $T\beta h^{nM18}$ virgin females, elevated OA levels induced postmating behaviors, including increased latency to copulation and ovipositor extrusion, along with a decrease in both male courtship and percentage copulating within 1 hour (Figure 1G, I-K). Notably, elevation of OA levels triggered egg laying even in the absence of copulation (Figure 1H). In most cases, supplying OA to $T\beta h^{nM18}$ mutants restored their behavior to that of controls without added OA (Figure 1G-J). These results support an adult-specific role for OA in female postmating behaviors.

Tdc2^{+} neurons are involved in female receptivity and postmating behaviors

Given that artificially increasing OA levels causes postmating responses in virgin females, we asked whether activation of OA producing neurons is sufficient to induce similar responses. We used the *Tdc2-Gal4* driver [11] to express the heat-activated cation channel gene *TrpA1* in octopaminergic-tyraminergic neurons (*Tdc2*$^{+}$ neurons), and assessed the behavioral effects of transiently activating these *Tdc2*$^{+}$ neurons. At 22°C, *Tdc2-Gal4/UAS-TrpA1* virgin females behaved indistinguishably from virgin controls (Figure 2A-C). However, at 32°C, *Tdc2-Gal4/UAS-TrpA1* virgins showed reduced levels of copulation within 1 hour, increased ovipositor extrusion, and increased egg-laying (Figure 2A-C), showing that transient activation of *Tdc2*$^{+}$ neurons induces postmating behaviors. In complementary experiments, we found that silencing *Tdc2*$^{+}$ neurons by expressing the synaptic vesicle exocytosis blocker gene, *tetanus toxin* (*TNT*), largely recapitulated the defects observed in *Tβh*^{nM18} mated females. *Tdc2-Gal4/UAS-TNT* mated females showed increased levels of remating (Figure 2D), reduced ovipositor extrusion (Figure 2E), and reduced egg-laying (Figure 2G) compared to mated controls. These mated females remained highly attractive to males, demonstrated by significantly higher levels of elicited courtship (Figure 2F). Given that activation of *Tdc2*$^{+}$ neurons induces postmating behaviors in virgin females, and that inhibition of these neurons disrupts postmating behaviors in mated females, we conclude that OA producing neurons are key for modulating female behaviors.

A small subset of sexually dimorphic neurons co-express *Tdc2* and *dsx* in the Abg

doublesex (*dsx*) is key for establishing sex-specific neural circuitry controlling male and female sexual behavior in flies [16, 17]. Indeed, ~27 *dsx*$^{+}$ neurons in the Abg have been implicated in the modulation of female postmating responses [9]. There are ~38 *Tdc2*$^{+}$ neurons along the ventral midline of the abdominal segment of the adult female ventral nerve cord (VNC; 38 ±3, n=10; Figure 3A), some of which project to the reproductive system [10, 11, 14]. We thus asked whether *Tdc2*$^{+}$ neurons were also *dsx* positive. To test this possibility, we implemented a FLP/FRT intersectional strategy [18] to subdivide the *dsx* circuitry into functionally defined subsets of neurons. We inserted the coding sequence of the FLP recombinase into the *dsx* locus by homologous recombination, creating a *dsx*^{FLP} allele that drives FLP-mediated recombination specifically in *dsx*$^{+}$ cells (Figure S1). We then combined the *dsx*^{FLP} line with *Tdc2-Gal4* and a Gal4/FLP-responsive membrane or nuclear reporter (*UAS>stop>mCD8::GFP* or *UAS>stop>nLacZ*, respectively). Both membrane and nuclear reporter expression was restricted to a small subset of approximately nine *Tdc2*/*dsx*$^{+}$ neurons within the female Abg (Figure 3B-D, respectively and Figure S2C,D). No *Tdc2/dsx*$^{+}$ cell bodies were found in the brain (Figure S2A,C and Figure S2E) or non-neuronal tissues (data not shown). *Tdc2*/*dsx*$^{+}$ neurons in the Abg project to specific locations in the reproductive system, innervating the lateral and common oviducts, uterus, and glandular parovaria, along with the seminal receptacle and spermathecae, the female sperm storage organs (Figure 3F-J). We also detected *Tdc2*/*dsx*$^{+}$ neurons innervating the longitudinal muscles in the sixth abdominal segment of the tergite (Figure S2F). Primary sex peptide (SP)-sensing neurons in the uterus can be identified by their location near the spermathecae and seminal receptacle and expression of the neuronal marker ELAV [9]. We established that *Tdc2*/*dsx*$^{+}$ neurons do not include these SP-sensing neurons by co-staining with ELAV (Figures 3H1-H3).

To evaluate whether *Tdc2/dsx*$^{+}$ neurons were sexually dimorphic, we characterized the intersecting neurons in the male central nervous system. As in females, no neurons were found in the brain (Figure S2B), but we detected a marked dimorphism in *Tdc2/dsx*$^{+}$ neuronal numbers in the male Abg (males: 3.3 ± 0.1 n=20, females: 9.1 ± 0.7, n=24) (Figure 3C). *Tdc2/dsx*$^{+}$ neurons project to the male reproductive system, innervating the ejaculatory duct, testes and accessory glands (Figure S2H-J). We also found *Tdc2/dsx*$^{+}$ neurons innervating the longitudinal muscles in the sixth abdominal segment of the male tergite (Figure S2G). These results show clear sexual dimorphism in *Tdc2/dsx*$^{+}$ neuronal cell numbers. We further confirmed this finding by expressing a membrane reporter (*UAS-mCD8::GFP*) using *dsx*^{Gal4} and labeling *Tdc2*$^{+}$ neurons with a Tdc2-specific antibody in the female and male brain and VNC (Figure S3A-F). Interestingly, we did not find any *Tdc2*$^{+}$ neurons co-expressing *fruitless*^{Gal4} (*fru*^{Gal4}) or *pickpocket* (*ppk*) in the female Abg (Figure S3G and S3H,I, respectively), suggesting that these neurons are *fru/ppk-*. Thus, our data support a role for *Tdc2/dsx*$^{+}$ neurons in the modulation of sex-specific behaviors.

Tdc2^{+}*/dsx*$^{+}$ circuitry modulates postmating behaviors

To test whether *Tdc2/dsx*$^{+}$ neurons are required for postmating behavioral responses, we activated these intersected neurons by expressing *TrpA1*. *Tdc2-Gal4/UAS>stop>TrpA1; dsx*^{FLP} virgin females behaved indistinguishably from virgin controls at 22°C (Figure 4A-C). However, thermal activation of *Tdc2/dsx*$^{+}$ neurons reduced receptivity, as only a small percentage of females copulated within 1 hour (Figure 4A). Moreover, these females showed increased ovipositor extrusion and egg-laying (Figure 4B,C). The opposite manipulation, silencing these *Tdc2/dsx*$^{+}$ neurons with TNT, had opposite effects on behavior. *Tdc2-Gal4/UAS>stop>TNT; dsx*^{FLP} mated females showed disrupted postmating responses; these females were more receptive than wild-type mated females, as they remated at a significantly higher frequency (Figure 4D) and showed decreased levels of ovipositor extrusion (Figure 4E). In addition, they elicited more male courtship and showed decreased levels of egg-laying (Figure 4F,G). Notably, activating or silencing this restricted set of approximately nine *Tdc2/dsx*$^{+}$ neurons in the Abg is sufficient to reproduce the behaviors observed when *TNT* or *TrpA1* are expressed in all *Tdc2*$^{+}$ neurons (Figure 2) or the previously identified ~27 *dsx/ET*$^{FLP250+}$ neurons [9]. Minor differences in the magnitude of the effects may be due to different strengths of expression with different drivers and from transgenes with and without the FLP-out stop cassette. In addition, we employed RNAi to knockdown *Tdc2* expression in *dsx*$^{+}$ cells and found that *dsx*^{Gal4}*/UAS-Tdc2RNAi* mated females showed increased levels of receptivity compared with controls (28.5% remated versus <4% of mated controls; n=30; $p<0.05$), supporting a role for OA signaling in *dsx*$^{+}$ neurons in mediating the postmating response.

Our study has identified sexually dimorphic *Tdc2/dsx*$^{+}$ circuitry responsible for modulating female postmating behaviors, such as reduced receptivity, increased levels of rejection, and egg-deposition. We found that *Tdc2/dsx*$^{+}$ neurons are sexually dimorphic in cell numbers, suggesting the presence of unique neuronal elements in one sex versus the other modulate sex-specific behaviors. Given that increasing OA in virgin females is sufficient to initiate postmating responses, we propose that copulation triggers OA release in the female reproductive system, which in turn modulates postmating responses. Our anatomical data

show that *Tdc2*$^+$ neurons located at the distal tip of the VNC that innervate the reproductive tract are *dsx*$^+$; therefore, egg-laying is likely to be directly modulated by *Tdc2/dsx*$^+$ neurons acting on muscles as previously described [13–15, 19, 20]. Interestingly, *ppk/dsx/fru*$^+$ neurons in the uterus, previously identified as SP-sensing neurons [7–9], project toward the female Abg [7], where *Tdc2*$^+$ neurons are located (Figure S3), raising the possibility that *Tdc2/dsx*$^+$ neurons act downstream of SP signaling.

Receptivity is presumably a more complex behavior than egg-laying, and the mechanisms downstream of *Tdc2/dsx*$^+$ neurons regulating receptivity are most likely independent of those regulating egg-laying. Indeed, egg production is not required for decreased receptivity after mating [21], egg-laying and receptivity are differentially affected by manipulations of *fru*$^+$, *dsx*$^+$, and *ppk*$^+$ neurons [22], and heterozygous *Tβh*^{nM18}/+ females showed increased receptivity but normal egg-laying (Figure 1). These results suggest that receptivity and egg-laying behaviors are differentially regulated by *Tdc2/dsx*$^+$ neurons.

Early gynandromorph studies mapped a region of the dorsal brain required for female receptivity [23]. *Tdc2/dsx*$^+$ neurons do not project to the brain, suggesting that if the brain is required for decreased receptivity after mating, OA signaling must modulate other neurons connected with decision centers in the brain [24]. Candidate targets of OA neuromodulation by *Tdc2/dsx*$^+$ neurons include descending projections from the brain to the Abg, ascending projections from the Abg to the brain, local circuits in the Abg, or SP sensing neurons in the uterus. Interestingly, *Tdc2/dsx*$^+$ neurons innervate the seminal receptacle and spermathecae, which store sperm, and the glandular parovaria (Figure 3), which secrete proteins required for sperm maturation and function [25]. SP binds to sperm and is kept in the females' spermathecae for several days after being transferred from the male during mating [1]. SP's slow release from stored sperm may allow it to gradually access its target cells to induce postmating responses [5]. This raises the interesting possibility that *Tdc2/dsx*$^+$ neurons may indirectly modulate SP sensing neurons by regulating sperm release and/or secretion of sperm-capacitating proteins. We propose that OA has a neuromodulatory role in coordinating sperm availability, egg release and reduction of receptivity after copulation.

Our findings represent an important step forward in the delineation of the neuronal circuitry required in females to adapt their behavior and physiology in response to reproductive state.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Caroline Fabre, Chris Elliott, Yael Heifetz, Sean Sweeney, Maria de la Paz Fernández and Stuart Wigby for comments on the manuscript. We are also grateful to Barry Dickson, Paul Garrity, Jay Hirsh, Maria Monastirioti, Gerry Rubin, Sean Sweeney and Wesley Grueber for providing valuable reagents. This work was supported by grants from the BBSRC (BB/I021817/1) to C.R and T.N., NERC (NE/J023647/1) and Wellcome Trust (WT082987MF) to M.C.N., and EPA Abraham Cephalosporin Trust Fund Fellowship to C.R.

References

1. Kubli E. Sex-peptides: seminal peptides of the Drosophila male. Cell Mol Life Sci. 2003; 60: 1689–1704. [PubMed: 14504657]
2. Avila FW, Sirot LK, LaFlamme BA, Rubinstein CD, Wolfner MF. Insect seminal fluid proteins: identification and function. Annu Rev Entomol. 2011; 56: 21–40. [PubMed: 20868282]
3. Chen PS, Stumm-Zollinger E, Aigaki T, Balmer J, Bienz M, Bohlen P. A male accessory gland peptide that regulates reproductive behavior of female D. melanogaster. Cell. 1988; 54: 291–298. [PubMed: 3135120]
4. Aigaki T, Fleischmann I, Chen PS, Kubli E. Ectopic expression of sex peptide alters reproductive behavior of female D. melanogaster. Neuron. 1991; 7: 557–563. [PubMed: 1931051]
5. Liu H, Kubli E. Sex-peptide is the molecular basis of the sperm effect in Drosophila melanogaster. Proc Natl Acad Sci U S A. 2003; 100: 9929–9933. [PubMed: 12897240]
6. Chapman T, Bangham J, Vinti G, Seifried B, Lung O, Wolfner MF, Smith HK, Partridge L. The sex peptide of Drosophila melanogaster: female post-mating responses analyzed by using RNA interference. Proc Natl Acad Sci U S A. 2003; 100: 9923–9928. [PubMed: 12893873]
7. Hasemeyer M, Yapici N, Heberlein U, Dickson BJ. Sensory neurons in the Drosophila genital tract regulate female reproductive behavior. Neuron. 2009; 61: 511–518. [PubMed: 19249272]
8. Yang CH, Rumpf S, Xiang Y, Gordon MD, Song W, Jan LY, Jan YN. Control of the postmating behavioral switch in Drosophila females by internal sensory neurons. Neuron. 2009; 61: 519–526. [PubMed: 19249273]
9. Rezaval C, Pavlou HJ, Dornan AJ, Chan YB, Kravitz EA, Goodwin SF. Neural circuitry underlying Drosophila female postmating behavioral responses. Curr Biol. 2012; 22: 1155–1165. [PubMed: 22658598]
10. Avila FW, Bloch Qazi MC, Rubinstein CD, Wolfner MF. A requirement for the neuromodulators octopamine and tyramine in Drosophila melanogaster female sperm storage. Proc Natl Acad Sci U S A. 2012; 109: 4562–4567. [PubMed: 22393023]
11. Cole SH, Carney GE, McClung CA, Willard SS, Taylor BJ, Hirsh J. Two functional but noncomplementing Drosophila tyrosine decarboxylase genes: distinct roles for neural tyramine and octopamine in female fertility. J Biol Chem. 2005; 280: 14948–14955. [PubMed: 15691831]
12. Monastirioti M, Linn CE Jr, White K. Characterization of Drosophila tyramine beta-hydroxylase gene and isolation of mutant flies lacking octopamine. J Neurosci. 1996; 16: 3900–3911. [PubMed: 8656284]
13. Monastirioti M. Distinct octopamine cell population residing in the CNS abdominal ganglion controls ovulation in Drosophila melanogaster. Dev Biol. 2003; 264: 38–49. [PubMed: 14623230]
14. Middleton CA, Nongthomba U, Parry K, Sweeney ST, Sparrow JC, Elliott CJ. Neuromuscular organization and aminergic modulation of contractions in the Drosophila ovary. BMC Biol. 2006; 4: 17. [PubMed: 16768790]
15. Rodriguez-Valentin R, Lopez-Gonzalez I, Jorquera R, Labarca P, Zurita M, Reynaud E. Oviduct contraction in Drosophila is modulated by a neural network that is both, octopaminergic and glutamatergic. J Cell Physiol. 2006; 209: 183–198. [PubMed: 16826564]
16. Villella A, Hall JC. Neurogenetics of courtship and mating in Drosophila. Adv Genet. 2008; 62: 67–184. [PubMed: 19010254]
17. Pavlou HJ, Goodwin SF. Courtship behavior in Drosophila melanogaster: towards a ‘courtship connectome’. Curr Opin Neurobiol. 2013; 23: 76–83. [PubMed: 23021897]
18. Bohm RA, Welch WP, Goodnight LK, Cox LW, Henry LG, Gunter TC, Bao H, Zhang B. A genetic mosaic approach for neural circuit mapping in Drosophila. Proc Natl Acad Sci U S A. 2010; 107: 16378–16383. [PubMed: 20810922]
19. Rubinstein CD, Wolfner MF. Drosophila seminal protein ovulin mediates ovulation through female octopamine neuronal signaling. Proc Natl Acad Sci U S A. 2013; 110: 17420–17425. [PubMed: 24101486]
20. Lee HG, Seong CS, Kim YC, Davis RL, Han KA. Octopamine receptor OAMB is required for ovulation in Drosophila melanogaster. Dev Biol. 2003; 264: 179–190. [PubMed: 14623240]

21. Barnes AI, Boone JM, Partridge L, Chapman T. A functioning ovary is not required for sex peptide to reduce receptivity to mating in D. melanogaster. J Insect Physiol. 2007; 53: 343–348. [PubMed: 17303161]

22. Haussmann IU, Hemani Y, Wijesekera T, Dauwalder B, Soller M. Multiple pathways mediate the sex-peptide-regulated switch in female Drosophila reproductive behaviours. Proceedings Biological sciences / The Royal Society. 2013; 280 20131938

23. Szabad J, Fajszi C. Control of female reproduction in Drosophila: genetic dissection using gynandromorphs. Genetics. 1982; 100: 61–78. [PubMed: 6807751]

24. Farooqui T. Octopamine-mediated neuromodulation of insect senses. Neurochemical research. 2007; 32: 1511–1529. [PubMed: 17484052]

25. Allen AK, Spradling AC. The Sf1-related nuclear hormone receptor Hr39 regulates Drosophila female reproductive tract development and function. Development. 2008; 135: 311–321. [PubMed: 18077584]

Europe PMC Funders Author Manuscripts

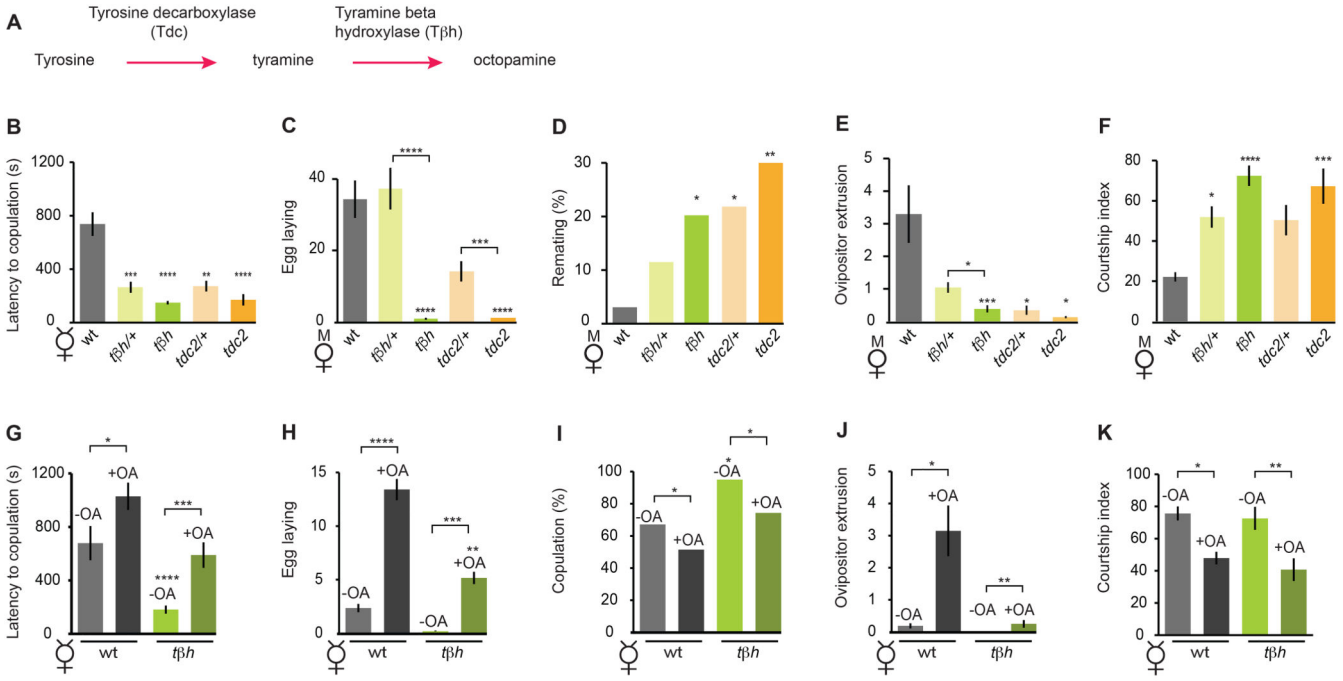

Figure 1. Octopamine regulates female receptivity and postmating responses.

(A) The OA biosynthesis cascade.

(B) Lack of OA increases receptivity in virgin females. *Tβh* (*Tβh*^{nM18}) and *Tdc2* (*Tdc2*^{RO54}) mutant virgin females show increased receptivity, measured as latency to copulation (in seconds); n = 25-35.

(C-F) Lack of OA disrupts postmating responses in mated females. *Tβh* (*Tβh*^{nM18}) and *Tdc2* (*Tdc2*^{RO54}) mutant mated females show disrupted postmating responses.

(C) Number of eggs laid per female 48 hr after copulation; n = 20-35.

(D) Remating frequency for females tested 48 hr after the initial mating; n = 40-60.

(E) Female ovipositor extrusion per minute during courtship; n =18-35.

(F) Male courtship index of wild-type males paired with females of the indicated genotypes; n = 15-25.

(G-K) Increasing OA levels reduces receptivity and triggers postmating responses in wild-type and *Tβh* (*Tβh*^{nM18}) mutant virgin females.

(G) Latency to copulation (in seconds); n = 30-40.

(H) Number of eggs laid per female after 6 days; n = 45-55.

(I) Percentage of females that copulated within 1 hr; n = 35-45.

(J) Female ovipositor extrusion per minute during courtship; n= 16-20.

(K) Male courtship index of wild-type males paired with females of the indicated genotypes; n = 18-22.

CS flies were used as wild-type in all behavioral tests. Females labeled +OA were fed 7.5 mg/mL of OA. Error bars indicate SEM. Statistical comparisons of the indicated genotypes were made against CS (B-F) or OA$^-$ wild-type controls (G-K), unless otherwise indicated. A Kruskal-Wallis ANOVA test was performed in B,C,E-H and J,K, and Fisher exact test in D, I. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Virgin and mated females are indicated as V and M, respectively.

Europe PMC Funders Author Manuscripts

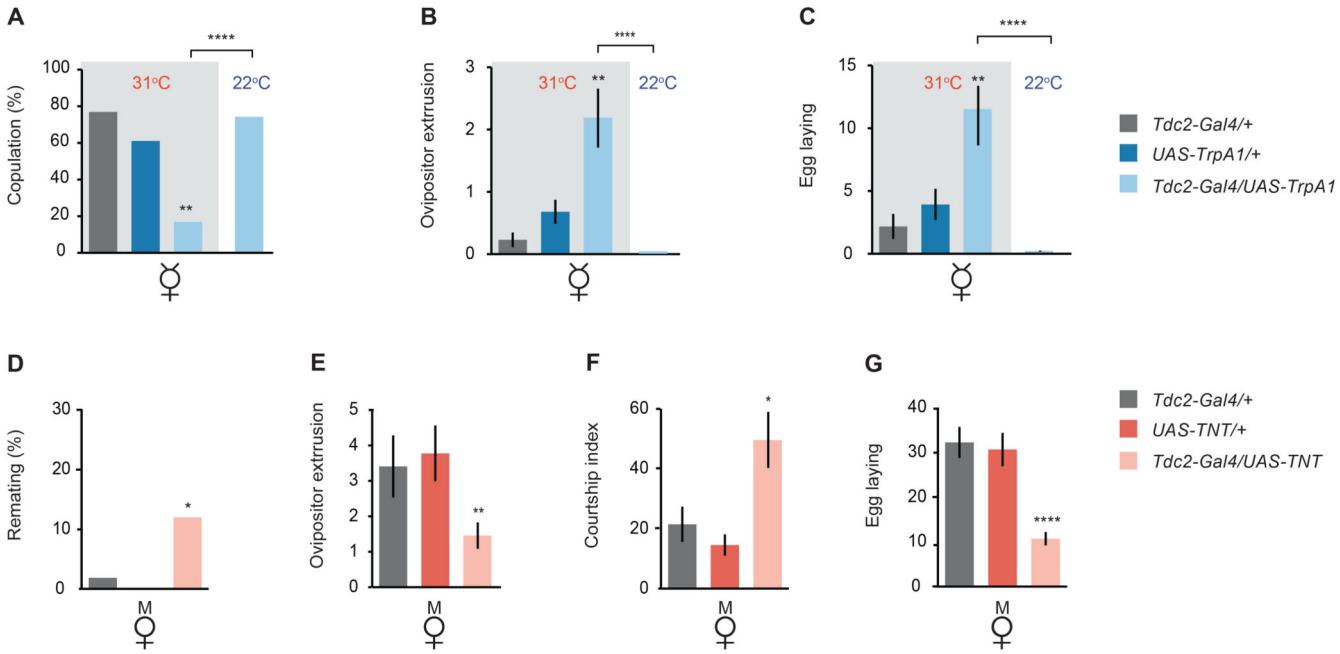

Figure 2. *Tdc2*$^{+}$ neurons are involved in postmating behaviors.

(A-C) Artificially activating *Tdc2*$^{+}$ neurons decreases receptivity in virgin females.

(A) Percentage of females that copulated within 1 hr; n = 35-45.

(B) Female ovipositor extrusion per minute during courtship; n = 25-30.

(C) Number of eggs laid per female during 48 hr; n = 35-45.

(D-G) Silencing *Tdc2*$^{+}$ neurons disrupts postmating responses in mated females.

(D) Remating frequency for females tested 48 hr after the initial mating; n = 30-40.

(E) Female ovipositor extrusion per minute during courtship; n = 12-16.

(F) Male courtship index of wild-type males paired with females of the indicated genotypes; n = 15-18.

(G) Number of eggs laid per female 48 hr after copulation; n = 25-35.

Error bars indicate ±SEM. Statistical comparisons were made against *Tdc2-Gal4*/+ (A-G) and *UAS-TrpA1*/+ in (A-C) or *UAS-TNT*/+ (in D-G), unless otherwise indicated. Kruskal-Wallis ANOVA test was performed in B,C,E-G and Fisher exact test in A,D. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Virgin and mated females are indicated as V and M, respectively. Note that for *Tdc2-Gal4/UAS-Trpa1* at 22°C in (B-C) and for *UAS-TNT*/+ in (D), error bars are not visible as they are close to 0.

Europe PMC Funders Author Manuscripts

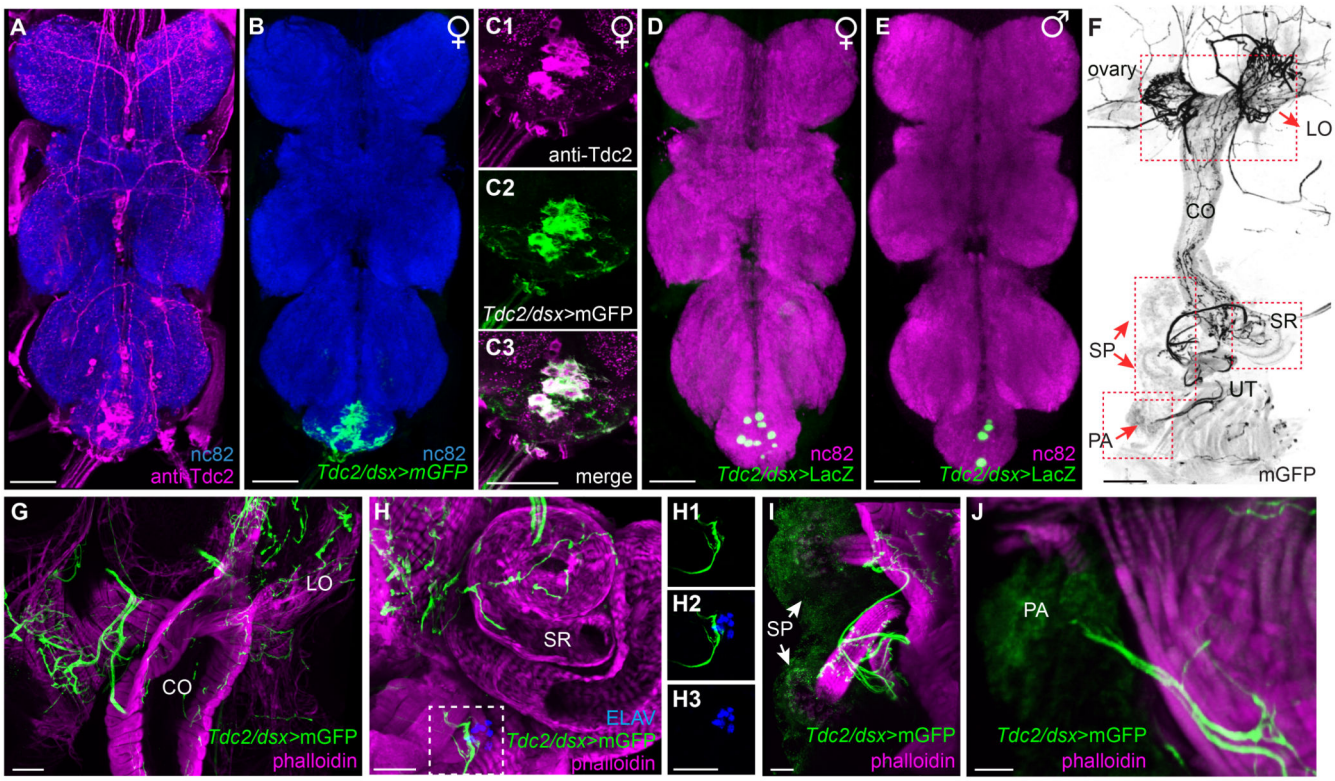

Figure 3. Identification of *Tdc2*$^{+}$/*dsx*$^{+}$ co-expressing neurons in the nervous system.

Combination of the *dsx*^{FLP} line with *Tdc2-Gal4* allows expression of Gal4/FLP-responsive *UAS>stop>reporters* in *Tdc2/dsx*$^{+}$-coexpressing neurons.

(A-C) Visualization of *Tdc2*$^{+}$ and *Tdc2/dsx*$^{+}$ cell bodies and projections in the female VNC. *Tdc2*$^{+}$ neurons are visualized with anti-Tdc2 antibody (magenta) in the female VNC (A). Approximately nine *Tdc2/dsx*$^{+}$ cell bodies are labeled with *UAS>stop>mCD8::GFP* reporter (*Tdc2/dsx*>mGFP; green) in the female VNC (B and C). Higher magnification of the female Abg in (B) depicting *Tdc2/dsx*$^{+}$ neurons (green) colabeled with anti-Tdc2 antibody (magenta) is shown in (C1)-(C3). In (A) and (B), neuropil is counterstained with anti-nC82 (blue).

(D and E) Visualization of *Tdc2/dsx*$^{+}$ neuronal nuclei expressing the *UAS>stop>nLacZ* reporter (*Tdc2/dsx*>LacZ; green). Approximately nine *Tdc2/dsx*$^{+}$ nuclei are detected in the female VNC (D) and approximately three in the male VNC (E). Anti β-Gal is shown in green. Neuropil is counterstained with anti-nC82 (magenta).

(F-J) Visualization of *Tdc2/dsx*$^{+}$ cell bodies and projections in the female reproductive system.

(F) Female reproductive system showing *Tdc2/dsx*$^{+}$ innervations (*Tdc2/dsx* > mGFP; black) in the lateral oviducts (LO), common oviduct (CO), uterus (UT), spermathecae (SP), seminal receptacle (SR), and parovaria (PA) (indicated by red arrows).

(G-J) Higher magnification of lateral and common oviducts (G), seminal receptacle (H), spermathecae (I), and parovaria (J). *Tdc2/dsx*$^{+}$ neuronal projections are shown in green (*Tdc2/dsx*>mGFP) and phalloidin (a marker for F-actin) in magenta. Lack of colocalization between *Tdc2/dsx*$^{+}$ neuronal cell bodies and SP sensory nuclei (white box in H) is shown at

higher magnification in (H1)-(H3). *Tdc2/dsx*$^{+}$ neurons are shown in green, and SP sensory neurons are stained with the neuronal nuclear marker anti-ELAV (blue).
Scale bars represent 50 μm (A, B, D, and E), 100 μm (F), and 25 μm (C and G−J). See also Figures S2 and S3.

Europe PMC Funders Author Manuscripts

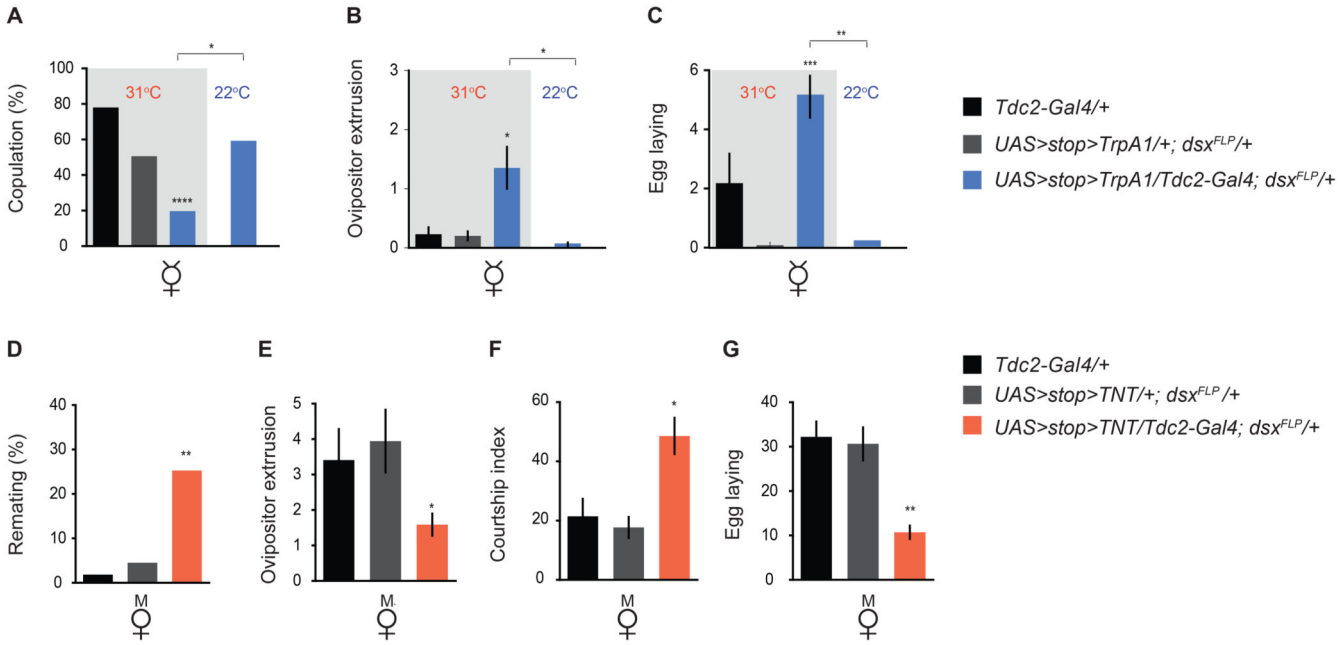

Figure 4. A subset of sexually dimorphic *Tdc2/dsx*$^{+}$ neurons is required for female postmating behavioral responses.

Combination of the *dsx*^{FLP} line with *Tdc2-Gal4* allows expression of the selected effectors, *UAS>stop>TrpA1* (A-C) or *UAS>stop>TNT* (D-G) in all intersecting neurons (*dsx∩Tdc2).*

(A-C) Artificial activation of *Tdc2/dsx*$^{+}$ neurons reduces receptivity and increases postmating responses in virgin females.

(A) Receptivity was scored as percentage of females that copulated within 1 hr. n = 45-55.

(B) Female ovipositor extrusion per minute during courtship. n = 23-33.

(C) Number of eggs laid per female in 48 hr after copulation. n = 25-45.

(D-G) Silencing of *Tdc2/dsx*$^{+}$ neurons reduces postmating responses in mated females.

(D) Remating frequency for females tested 48 hr after the initial mating. n = 30-40.

(E) Female ovipositor extrusion per minute during courtship. n = 14-24.

(F) Male courtship index of wild-type males paired with females of the indicated genotypes. n = 35-45.

(G) Number of eggs laid per female 48 hr after copulation. n = 25-35.

Error bars indicate ± SEM. A Kruskal-Wallis ANOVA test was performed in B,C and E-G and Fisher's exact test in A,D. Statistical comparisons were made against *Tdc2-Gal4*/+ (A-G) and *UAS>stop>TrpA1/+;dsx*^{FLP}/+ (A−C) or *UAS>stop>TNT/+;dsx*^{FLP}/+ (D-G), unless otherwise indicated. *p < 0.05, **p < 0.01, and ***p < 0.001. Virgin and mated females are indicated as V and M, respectively.

Europe PMC Funders Author Manuscripts