end-to-side or side-to-side filament interactions?

By slightly modifying the parameters of the experimental model, one can begin to investigate these questions. For example, Shs1 is a prime candidate for further study, as it has previously been shown to substitute for the terminal Cdc11 in the octamer complex. *In vitro*, the substitution of Cdc11 with Shs1 results in the dramatic formation of ring and mesh structures in conditions that originally favoured single octamers and filaments [14]. If this single subunit change is sufficient to generate distinct septin structures, then it is possible that the 13 mammalian septin genes (many of which encode for multiple isoforms) are able to form a large repertoire of unique structures. This work opens many avenues that allow for future investigation of the roles of factors such as local lipid concentration, association with septin binding proteins and post-translational modification in the formation of the wide variety of septin structures.

References

1. Mostowy, S., and Cossart, P. (2012). Septins: the fourth component of the cytoskeleton. Nat. Rev. Mol. Cell Biol. *13*, 183–194.
2. DeMay, B.S., Noda, N., Gladfelter, A.S., and Oldenbourg, R. (2011). Rapid and quantitative imaging of excitation polarized fluorescence reveals ordered septin dynamics in live yeast. Biophys. J. *101*, 985–994.
3. Dobbelaere, J., and Barral, Y. (2004). Spatial coordination of cytokinetic events by compartmentalization of the cell cortex. Science *305*, 393–396.
4. Bridges, A.A., Zhang, H., Mehta, S.B., Occhipinti, P., Tani, T., and Gladfelter, A.S. (2014). Septin assemblies form by diffusion-driven annealing on membranes. Proc. Natl. Acad. Sci. USA *111*, 2146–2151.
5. Bertin, A., McMurray, M.A., Pierson, J., Thai, L., McDonald, K.L., Zehr, E.A., García, G., Peters, P., Thorner, J., and Nogales, E. (2012). Three-dimensional ultrastructure of the septin filament network in Saccharomyces cerevisiae. Mol. Biol. Cell *23*, 423–432.
6. Kinoshita, M., Field, C.M., Coughlin, M.L., Straight, A.F., and Mitchison, T.J. (2002). Self- and actin-templated assembly of mammalian septins. Dev. Cell *3*, 791–802.
7. Bertin, A., McMurray, M.A., Grob, P., Park, S.-S., García, G., Patanwala, I., Ng, H.-L., Alber, T., Thorner, J., and Nogales, E. (2008). Saccharomyces cerevisiae septins: supramolecular organization of heterooligomers and the mechanism of filament assembly. Proc. Natl. Acad. Sci. USA *105*, 8274–8279.
8. Sirajuddin, M., Farkasovsky, M., Hauer, F., Kühlmann, D., Macara, I.G., Weyand, M., Stark, H., and Wittinghofer, A. (2007). Structural insight into filament formation by mammalian septins. Nature *449*, 311–315.
9. Zhang, J., Kong, C., Xie, H., McPherson, P.S., Grinstein, S., and Trimble, W.S. (1999). Phosphatidylinositol polyphosphate binding to the mammalian septin H5 is modulated by GTP. Curr. Biol. *9*, 1458–1467.
10. Golebiewska, U., Kay, J.G., Masters, T., Grinstein, S., Im, W., Pastor, R.W., Scarlata, S., and McLaughlin, S. (2011). Evidence for a fence that impedes the diffusion of phosphatidylinositol 4,5-bisphosphate out of the forming phagosomes of macrophages. Mol. Biol. Cell *22*, 3498–3507.
11. Tanaka-Takiguchi, Y., Kinoshita, M., and Takiguchi, K. (2009). Septin-mediated uniform bracing of phospholipid membranes. Curr. Biol. *19*, 140–145.
12. Kim, M.S., Froese, C.D., Estey, M.P., and Trimble, W.S. (2011). SEPT9 occupies the terminal positions in septin octamers and mediates polymerization-dependent functions in abscission. J. Cell Biol. *195*, 815–826.
13. Sellin, M.E., Sandblad, L., Stenmark, S., and Gullberg, M. (2011). Deciphering the rules governing assembly order of mammalian septin complexes. Mol. Biol. Cell *22*, 3152–3164.
14. García, G., Bertin, A., Li, Z., Song, Y., McMurray, M.A., Thorner, J., and Nogales, E. (2011). Subunit-dependent modulation of septin assembly: budding yeast septin Shs1 promotes ring and gauze formation. J. Cell Biol. *195*, 993–1004.
15. Brill, J.A., Wong, R., and Wilde, A. (2011). Phosphoinositide function in cytokinesis. Curr. Biol. *21*, R930–R934.
16. Booth, J.W., Trimble, W.S., and Grinstein, S. (2001). Membrane dynamics in phagocytosis. Sem. Immunol. *13*, 357–364.
17. Joo, E., Surka, M.C., and Trimble, W.S. (2007). Mammalian SEPT2 is required for scaffolding nonmuscle myosin II and its kinases. Dev. Cell *13*, 677–690.
18. Huang, Y.-W., Yan, M., Collins, R.F., Diciccio, J.E., Grinstein, S., and Trimble, W.S. (2008). Mammalian septins are required for phagosome formation. Mol. Biol. Cell *19*, 1717–1726.

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Neural Circuits: Anatomy of a Sexual Behavior

Females of many species, once mated, undergo a rapid change in reproductive physiology and behavior, shifting from a sexually receptive state to one devoted to the rearing of offspring. Two recent reports shed light on the neural circuitry governing the female post-mating response in the fruit fly *Drosophila*, providing insight into the neurobiological processes governing a complex behavior.

Joshua J. Krupp and Joel D. Levine

"There are lots of things that you can do alone", but when it comes to courtship and mating, as the song goes, "it takes two to tango". If one partner is not disposed to dance, well, the other partner is just plain out of luck. Recent advances in the neurobiology of reproductive behavior of *Drosophila* have begun to address the steps (and missteps) of this 'tango' — from a standpoint of both the male and female of the species.

Drosophila courtship is like a dance involving visual, auditory, and chemical cues [1]. Although the male is the more flamboyant of the partners, the female is the one that 'leads' and often determines whether mating will occur. Females unreceptive to mating exhibit rejection behaviors including kicking and ovipositor extrusion, whereas receptive females facilitate copulation by slowing down and ceasing rejection behaviors. While virgin females are highly receptive and rapidly copulate with a suitable partner, mated females undergo a rapid change in their physiology and behavior, becoming temporarily sexually unreceptive to further copulation, while increasing feeding, ovulation and egg-laying [2]. It is thought that instead of investing time and energy in potentially risky and unnecessary behavior (by mating multiple times), once mated, females shift to behaviors that promote the generation of offspring. This shift in female reproductive physiology and behavior is called the post-mating response.

The post-mating response is thought to be triggered primarily by sex peptide (SP), a small peptide synthesized by the male and transferred to the female during insemination [2]. Females mated to SP-deficient males re-mate when assayed 24-hours after their first mating [2], while virgin females injected with SP are rendered unreceptive by the next day [3]. A SP-responsive G protein-coupled receptor for

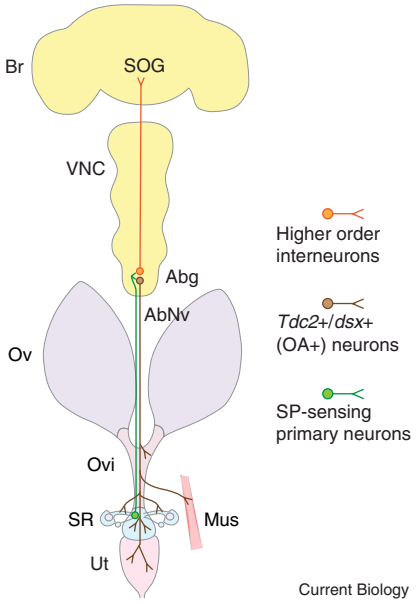

Figure 1. Partial map of the neural circuitry necessary for mediating female post-mating responses.

Octopamine expressing *Tdc2/dsx+* Abg neurons, identified by Rezával *et al.*, project to specific locations of the female reproductive system and are necessary for post-mating changes in sexual receptivity and egg-laying (brown neuron). How do these neurons regulate these responses? *Tdc2/dsx+* neurons are not found outside of the Abg and do not project to the brain, suggesting these neurons likely mediate female behavior by signaling to higher order interneurons (orange neuron), which project to the processing centers in the brain that direct behavior [7]. Alternatively, the *Tdc2/dsx+* neurons may act within a reflex arc, directly responding to sensory input and signaling back to the reproductive tract. As Rezával *et al*. [9] note, neurons previously identified as SP sensing neurons in the uterus (green neuron) project to the female Abg near to where the *Tdc2/dsx+* neurons are located [5], raising the possibility that *Tdc2/dsx+* neurons may directly act downstream of SP signaling to mediate aspects of the post-mating response. Br, brain; SOG, subesophageal ganglion; VNC, ventral nerve cord; Abg, abdominal ganglion; AbNv, abdominal nerve; Ov, ovary; Ovi, oviduct; SR, seminal receptacle, spermathecae, and parovaria; Ut, uterus; Mus, longitudinal muscle of 6th abdominal tergite.

SP-mediated post-mating responses has been identified [4]. Females lacking this sex peptide receptor (SPR) remain receptive, exhibiting virgin-like behaviors, post-mating. It has been shown that post-mating responses can be mediated by SPR activation in a set of six to eight sensory neurons located on the reproductive tract [5–7]. The neural circuitry acting downstream of SP signaling and the mechanisms responsible for modulating female behavior are now coming to light.

Two papers published recently in *Current Biology* [8,9] explore the neural components of female reproductive behavior. In the first report, Stephen Goodwin's group [8] takes an important step forward in defining the neural circuitry downstream of SP-responsive sensory neurons responsible for mediating aspects of the post-mating response in female reproductive behavior. In the second, Mariana Wolfner and Yael Heifetz, in collaboration with Yuval Garini's group [9], while also examining the neural regulation of the female post-mating response, reveal a pattern of highly orchestrated changes within neuronal termini innervating the female reproductive tract following insemination; these dynamic changes in neurotransmitter content and other signaling factors likely facilitate aspects of ovulation and fertilization [9].

In an influential paper published in *Current Biology* several years ago, Rezával and colleagues [7] identified components of neural circuitry, defined by the sex determination gene *doublesex* (*dsx*), involved in mediating female reproductive behavior. The circuitry they identified included primary neurons required for sensing SP in the female reproductive system, and downstream effectors of the SP-sensory neuronal pathway housed within the abdominal ganglion (Abg) of the ventral nerve cord (VNC). At the time, the authors proposed a compelling model in which, "female-specific *dsx* neurons play a central role in the coordinated regulation of behavioral and physiological responses after copulation — from reception of stimulatory inputs and transmission of these signals to higher-order centers for processing to generation of the associated behavioral motor outputs" [7]. In their new paper, Rezával *et al.* [8] refine their model by identifying a subpopulation of *dsx* Abg neurons that express the neurotransmitter octopamine; both the neurons and neurotransmitter are demonstrated to be critical for female post-mating behavior.

In *Drosophila*, the catecholamine octopamine regulates many aspects of female reproductive physiology, including ovulation [10]. Rezával *et al.* [8] first asked whether octopamine might also be required for female post-mating responses in sexual receptivity and egg-laying. Opposite to their normal post-mating responses, female flies deficient for octopamine remained highly receptive to courting males and laid few eggs following copulation. Conversely, virgin wild-type females, as well as octopamine-deficient females, normally highly receptive to suitable mates, when supplied with an exogenous source of octopamine rejected the advances of suitors and increased egg-laying without prior copulation. Importantly, experimental manipulation of the physiological activity of neurons that express the gene *Tdc2*, a gene encoding an enzyme required for octopamine biosynthesis, phenocopied the effects of octopamine, strongly suggesting that octopaminergic neuron(s) via octopamine signaling are both necessary for mediating adult-specific, post-mating responses in female reproductive behavior.

Rezával *et al.* [8] next turned their attention to refining the neural circuitry responsible for mediating the octopamine-dependent effects of female reproductive behavior. Like that of *dsx*, the expression of *Tdc2* (and by extension that of octopamine) is relatively widespread within the adult central nervous system (brain and ventral nerve cord). There are ~27 *dsx+* neurons and ~38 *Tdc2+* neurons in the VNC alone. Rezával *et al.* [8] reasoned that there may be significant overlap between *Tdc2+* (octopaminergic) and *dsx+* neurons given the similar role each population appears to play in mediating the post-mating response, and that within that overlap would reside a limited population of neurons critical for female behavior. Employing an elegant intersectional approach involving genetic mosaics the authors prove their hypothesis correct, identifying a sexually dimorphic population of ~9 *Tdc2/dsx+* neurons in females (~3 neurons in males) which via octopamine signaling are critical for mediating the post-mating response in female behavior (Figure 1) [8]. This simplifies (from a numbers standpoint) the neurons to focus on in future studies, and creates an important inroad to further map the circuit mediating female post-mating behavior.

The refined model of the neural circuitry governing the post-mating response contextualizes the neurobiological processes governing

reproductive physiology and behavior. The current model already has some interesting correlates with the accompanying report from Heifetz *et al.* [9]. Examining the neurobiology of the post-mating response, these authors uncovered systematic changes following copulation in the expression pattern of several neurochemicals, including octopamine, within the nerve terminals innervating the female reproductive tract.

Heifetz *et al.* [9] found that each region of the female reproductive tract displays a unique combination of neurochemicals, and that mating alters these combinations. The changes are rapid, occurring within minutes after the start of copulation, and continue to evolve several hours after completion, coinciding with reproductive tract maturation. These changes likely reflect dynamic alterations in the vesicular packaging and/or release of neurochemicals at synaptic sites. Although yet to be fully demonstrated, these highly orchestrated and progressive changes in neurotransmitter expression in all likelihood coordinate reproductive tract functions necessary for the post-mating increase in ovulation and fertilization. Determining if the neurons identified by Rezával *et al.* [8] are involved in mediating the changes in neurochemicals at the reproductive tract is an obvious, and accessible, place to continue the study of the neural components regulating female post-mating physiology and behavior.

Chemical signaling is an essential component of sexual behavior in *Drosophila*. The role of chemical signals extends from external cuticular hydrocarbon pheromones that communicate sex and species information and can either attract or repel potential mates [11], to internal signals such as the seminal peptides (which includes SP) transferred to the female during insemination and which effect sexual physiology and behavior [2]. That some of the changes reported by Heifetz *et al.* [9] in the neurochemistry of the neurons innervating the female reproductive tract seemingly occur independently of seminal proteins or sperm suggests that the physical process of mating itself may play a part in influencing the post-mating response — an interesting notion not yet explored in *Drosophila*. A related possibility and one that takes into account the importance of chemical signals is that male cuticular sex pheromones sensed by the female at close range during copulation exert a broader and longer-lasting influence on physiology and behavior than is currently recognized. With this interesting phenomenon not yet explained, it would appear that the neural circuit governing the post-mating response still holds new surprises waiting to be discovered.

Together, these reports [8,9] highlight an emerging focus on the ventral nerve cord in regulating the physiology and behavior of *Drosophila*. Like the vertebrate spinal cord, the ventral nerve cord of *Drosophila* is largely considered to be a thoroughfare for nerve fibers sending sensory input to higher brain centers and transmitting motor output to peripheral tissues. Recently, neurons of the VNC have been implicated in the regulation of tracheal branching [12], renal physiology [13], feeding and locomotion [14], sperm transfer [15], copulation duration [15,16], courtship song [17–19], female fertility [20], and the post-mating response [7]. These studies and others suggest that the VNC is an important site for integrating afferent sensory input from the periphery and efferent motor output from the brain. But like the spinal cord, the VNC may be able to do more than just that: as several of these studies suggest [16,19] it is possible that local neural circuits within the VNC may generate patterns of behavior in much the same way that central pattern generators (CPGs) are sufficient to direct locomotion, respiration, and swallowing in vertebrates. The functional properties and cellular mechanisms found throughout the *Drosophila* central nervous system are complex, intimately linked with sexual identity and, like the 'tango', may serve as a vehicle for interacting with others. Most important, they are increasingly tractable.

References

1. Villella, A., and Hall, J.C. (2008). Neurogenetics of courtship and mating in *Drosophila*. Adv. Genet. *62*, 67–184.
2. Kubli, E. (2003). Sex-peptides: seminal peptides of the *Drosophila* male. Cell. Mol. Life Sci. *60*, 1689–1704.
3. Chen, P.S., Stumm-Zollinger, E., Aigaki, T., Balmer, J., Bienz, M., and Böhlen, P. (1988). A male accessory gland peptide that regulates reproductive behavior of female *D. melanogaster*. Cell *54*, 291–298.
4. Yapici, N., Kim, Y.-J., Ribeiro, C., and Dickson, B.J. (2008). A receptor that mediates the post-mating switch in *Drosophila* reproductive behaviour. Nature *451*, 33–37.
5. Häsemeyer, M., Yapici, N., Heberlein, U., and Dickson, B.J. (2009). Sensory neurons in the *Drosophila* genital tract regulate female reproductive behavior. Neuron *61*, 511–518.
6. Yang, C.-H., Rumpf, S., Xiang, Y., Gordon, M.D., Song, W., Jan, L.Y., and Jan, Y.-N. (2009). Control of the postmating behavioral switch in *Drosophila* females by internal sensory neurons. Neuron *61*, 519–526.
7. Rezával, C., Pavlou, H.J., Dornan, A.J., Chan, Y.-B., Kravitz, E.A., and Goodwin, S.F. (2012). Neural circuitry underlying *Drosophila* female postmating behavioral responses. Curr. Biol. *22*, 1155–1165.
8. Rezával, C., Nojima, T., Neville, M.C., Lin, A.C., and Goodwin, S.F. (2014). Sexually dimorphic octopaminergic neurons modulate female postmating behaviors in Drosophila. Curr. Biol. *24*, 725–730.
9. Heifetz, Y., Lindner, M., Garini, Y., and Wolfner, M.F. (2014). Mating regulates neuromodulator ensembles at nerve termini innervating the *Drosophila* reproductive tract. Curr. Biol. *24*, 731–737.
10. Rubinstein, C.D., and Wolfner, M.F. (2013). *Drosophila* seminal protein ovulin mediates ovulation through female octopamine neuronal signaling. Proc. Natl. Acad. Sci. USA *110*, 17420–17425.
11. Billeter, J.-C., Atallah, J., Krupp, J.J., Millar, J.G., and Levine, J.D. (2009). Specialized cells tag sexual and species identity in *Drosophila melanogaster*. Nature *461*, 987–991.
12. Linneweber, G.A., Jacobson, J., Busch, K.E., Hudry, B., Christov, C.P., Dormann, D., Yuan, M., Otani, T., Knust, E., de Bono, M., *et al.* (2014). Neuronal control of metabolism through nutrient-dependent modulation of tracheal branching. Cell *156*, 69–83.
13. Talsma, A.D., Christov, C.P., Terriente-Felix, A., Linneweber, G.A., Perea, D., Wayland, M., Shafer, O.T., and Miguel-Aliaga, I. (2012). Remote control of renal physiology by the intestinal neuropeptide Pigment-Dispersing Factor in Drosophila. Proc. Natl. Acad. Sci. USA *109*, 12177–12182.
14. Mann, K., Gordon, M.D., and Scott, K. (2013). A pair of interneurons influences the choice between feeding and locomotion in *Drosophila*. Neuron *79*, 754–765.
15. Tayler, T.D., Pacheco, D.A., Hergarden, A.C., Murthy, M., and Anderson, D.J. (2012). A neuropeptide circuit that coordinates sperm transfer and copulation duration in Drosophila. Proc. Natl. Acad. Sci. USA *109*, 20697–20702.
16. Crickmore, M.A., and Vosshall, L.B. (2013). Opposing dopaminergic and GABAergic neurons control the duration and persistence of copulation in *Drosophila*. Cell *155*, 881–893.
17. Rideout, E.J., Billeter, J.-C., and Goodwin, S.F. (2007). The sex-determination genes *fruitless* and *doublesex* specify a neural substrate required for courtship song. Curr. Biol. *17*, 1473–1478.
18. Shirangi, T.R., Stern, D.L., and Truman, J.W. (2013). Motor control of *Drosophila* courtship song. Cell Rep. *5*, 678–686.
19. von Philipsborn, A.C., Liu, T., Yu, J.Y., Masser, C., Bidaye, S.S., and Dickson, B.J. (2011). Neuronal control of *Drosophila* courtship song. Neuron *69*, 509–522.
20. Castellanos, M.C., Tang, J.C.Y., and Allan, D.W. (2013). Female-biased dimorphism underlies a female-specific role for post-embryonic llp7 neurons in *Drosophila* fertility. Development *140*, 3915–3926.

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