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She's got nerve: Roles of octopamine in insect female reproduction

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Abstract

The biogenic monoamine octopamine (OA) is a crucial regulator of invertebrate physiology and behavior. Since its discovery in the 1950s in octopus salivary glands, OA has been implicated in many biological processes among diverse invertebrate lineages. It can act as a neurotransmitter, neuromodulator and neurohormone in a variety of biological contexts, and mediate processes including feeding, sleep, locomotion, flight, learning, memory, and aggression. Here, we focus on the roles of OA in female reproduction in insects. OA is produced in the octopaminergic neurons that innervate the female reproductive tract (RT). It exerts its effects by binding to receptors throughout the RT to generate tissue- and region-specific outcomes. OA signaling regulates oogenesis, ovulation, sperm storage, and reproductive behaviors in response to the female's internal state and external conditions. Mating profoundly changes a female's physiology and behavior. The female's OA signaling system interacts with, and is modified by, male molecules transferred during mating to elicit a subset of the post-mating changes. Since the role of OA in female reproduction is best characterized in the fruit fly *Drosophila melanogaster*, we focus our discussion on this species but include discussion of OA in other insect species whenever relevant. We conclude by proposing areas for future research to further the understanding of OA's involvement in female reproduction in insects.

Keywords

Octopamine; Reproduction; Fertility; Insect

Introduction

Octopamine (OA) is a biogenic monoamine central to invertebrate physiology and behavior. Originally identified in the salivary glands of *Octopus vulgaris* (Erspamer and Boretti 1951), OA is abundant in invertebrates but exists only as a trace amine with limited functions in vertebrates (Orchard 1982; Evans 1985; Roeder 1999; Borowsky et al. 2001; Berry 2004). OA and norepinephrine are structurally and functionally similar, and they are commonly regarded as counterparts in invertebrates and vertebrates, respectively. Across diverse species and organs, OA can function as a neurotransmitter, neuromodulator, and neurohormone (Orchard 1982; Roeder 1999; Roeder 2005; Farooqui 2012). Its roles in feeding, sleep,

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locomotion, flight, learning, memory, and aggression have been reviewed elsewhere (Roeder 2005; Farooqui 2012; Roeder 2020); in this article, we review OA's role in regulating female reproduction in insects, with a particular focus on the fruit fly, *Drosophila melanogaster*.

OA is synthesized from the amino acid tyrosine. The enzyme tyrosine decarboxylase (TDC) converts tyrosine to the biogenic amine tyramine (TA) by decarboxylation, and another enzyme, tyramine beta-hydroxylase (TBH), further converts TA to OA through hydroxylation (Fig 1A). Historically, TA was regarded as simply the intermediate molecule in OA synthesis, but recent studies suggest that TA has independent and sometimes antagonistic functions to OA in some biological contexts (Saraswati et al. 2004; Lange 2009a). For example, in *Drosophila melanogaster*, TA is involved in salt retention in the Malpighian tubules (Blumenthal 2003). In the honeybee, *Apis mellifera*, TA enhances ovary development of workers in the absence of the queen (Salomon et al. 2012; Matsuyama et al. 2015). In the blood-feeding bug, *Rhodnius prolixus*, TA regulates the spontaneous contractions of reproductive tissues of females (Hana and Lange 2017a). The *Drosophila* genome contains two TDC genes. *Tdc1* is expressed in non-neural tissues and *Tdc2* is expressed in the nervous system. The two genes share 52% nucleotide identity and 47% amino acid identity, with Tdc2 likely being a more active enzyme than Tdc1 (Monastirioti et al. 1996). Unlike the *Tdc*'s, *Tbh* is expressed almost exclusively in neural tissues (Monastirioti et al. 1996). This suggests that OA and TA are present in the nervous system, whereas non-neural tissues contain predominantly TA (Cole et al. 2005). Recent single-cell RNA-seq studies reveal that in the *Drosophila* brain and ventral nerve cord (VNC), most *Tdc2* expressing neurons also express *Tbh* (Davie et al. 2018; Allen et al. 2020; Cocanougher et al. preprint), although a high throughput fluorescence in situ hybridization (FISH) study shows limited *Tdc2* and *Tbh* colocalization in the dorsal and posterior brain (Meissner et al. 2019).

OA's effects on reproduction are mediated by the activity of OA neurons. The number of OA neurons range from ~100 in larger insects such as locusts (*Locusta migratoria* and *Schistocerca gregaria*) to ~40 in smaller insects such as *Drosophila melanogaster* (Roeder 2005; Farooqui 2012). In the *Drosophila* adult central nervous system (CNS), OA neurons are located throughout the brain and along the midline of the VNC (Monastirioti et al. 1995; Sinakevitch and Strausfeld 2006; Busch et al. 2009; Sherer et al. 2020). In the rest of the body, OA neurons innervate the antennae, legs, wings, halteres, skeletal muscles, *corpora allata*, heart and the reproductive tract (Pauls et al. 2018). Approximately 70% of OA neurons in *Drosophila* adult brain and VNC also co-transmit the neurotransmitter glutamate (Rodríguez-Valentín et al. 2006; Sherer et al. 2020); some OA-glutamate neurons in the VNC are likely motor neurons.

OA exerts its effects by binding to receptors on target cells. Being a neurotransmitter, neuromodulator, and neurohormone, OA can interact with receptors both local to its site of release and far away depending on the specific physiological context. OA receptors comprise a family of rhodopsin-like G-protein coupled receptors (Brody and Cravchik 2000), each consisting of 7 transmembrane (TM) domains, an extracellular N-terminus, and a cytosolic C-terminus (Strader et al. 1995). For biogenic amine receptors, like OA receptors, a conserved aspartic acid residue in TM3, serine residues in TM5, as well as

a phenylalanine in TM6 mediate ligand-receptor binding (Strader et al. 1995; Valdenaire and Vernier 1997; Huang et al. 2007). OA receptors are metabotropic, relaying signals via secondary messengers determined by the type of G-protein bound to the C-terminus and third intracellular loop (Chuang et al. 1996; Palczewski et al. 2000). There are three OA receptor classes: Octα1R, Octα2R, OctβR (Fig 1B) (Han et al. 1998; Balfanz et al. 2005; Evans and Maqueira 2005; Maqueira et al. 2005; Farooqui 2012; Qi et al. 2017). They are distinguished by their sequence similarity to either vertebrate α-adrenergic or β-adrenergic receptors (Evans and Maqueira 2005; Farooqui 2012), and the secondary messenger molecules they mobilize (Han et al. 1998; Grohmann et al. 2003; Balfanz et al. 2005; Maqueira et al. 2005; Ohtani et al. 2006; Wu et al. 2014; Qi et al. 2017) (Table 1). These classes of OA receptors have been identified and functionally characterized in *Drosophila melanogaster* and many other insects (Table 1) (Grohmann et al. 2003; Bischof and Enan 2004; Ohtani et al. 2006; Chen et al. 2010; Verlinden et al. 2010; Wu et al. 2012; Balfanz et al. 2014; Kastner et al. 2014; Wu et al. 2014; H.-M. Li et al. 2016; Wu et al. 2017; Hana and Lange 2017b; Huang et al. 2018; Wu et al. 2019). There are also receptors for OA's precursor molecule, TA, which are classified by their secondary messengers (Table 1) (Farooqui 2012). There is some evidence from *D. melanogaster* that Tyr1 (Oct-Tyr) receptors have affinity for both OA and TA (Robb et al. 1994; Reale et al. 1997). However, OA's activation of Tyr1 is less consistent in other insects, possibly as the result of different groups performing assays in different cell types (Blenau et al. 2000; Poels et al. 2001; Rotte et al. 2009; Farooqui 2012). Other *D. melanogaster* TA receptors are more sensitive to TA than OA (Bayliss et al. 2013; Huang et al. 2016). Here, we focus on the reproductive effects of receptors with a conserved, high affinity for OA. The diversity of OA receptor function highlights OA's ability to precisely modulate physiology and behavior, as a tissue's response to OA may be influenced by its complement of OA receptors.

Studies of OA's presence and function in female reproduction of diverse insect taxa have been made possible by various methods and techniques. Direct application of OA to *ex vivo* tissues (Cook and Peterson 1989; Middleton et al. 2006; Meiselman et al. 2018) or OA feeding to animals (Monastirioti et al. 1996; Cole et al. 2005; Rubinstein and Wolfner 2013) allows direct assessment of OA's role in biological processes. Immunohistochemistry and high-performance liquid chromatography (HPLC) facilitate detection of the location and concentration of OA (Monastirioti et al. 1995; Monastirioti et al. 1996; Monastirioti 2003; Heifetz et al. 2014). Additionally, electrophysiology can be used to examine the neuronal control of reproductive tissues (Clark and Lange 2003; Rodríguez-Valentín et al. 2006; Wong and Lange 2014). The abundance of genetic tools available in *Drosophila* have greatly contributed to the large and expanding body of knowledge in this traditional model system. The isolation and characterization of *Tbh* and *Tdc2* mutants enabled inquiry of OA's involvement in biological processes directly (Schüpbach and Wieschaus 1991; Monastirioti et al. 1996; Cole et al. 2005). The advent of the GAL4/UAS system made it possible to manipulate the expression of specific OA synthesis or receptor genes (Brand and Perrimon 1993; Monastirioti 2003; Cole et al. 2005; Lee et al. 2009; Lim et al. 2014; Deady and Sun 2015; Li et al. 2015). More recently, advancement in neurobiological tools enable fine-scale dissection of OA neuronal circuitry (Rodríguez-Valentín et al. 2006; Venken et al. 2011; Rubinstein and Wolfner 2013; Rezával et al. 2014; Meiselman et al. 2018; Masuzzo

et al. 2019; Yoshinari et al. 2020). Finally, the rise of 'omic technologies and CRISPR/Cas9-mediated gene editing unlocks new possibilities for research in *Drosophila* and other insect systems alike.

In this review, we detail OA's function in virgin and mated female reproductive physiology, from oogenesis to ovulation and sperm storage, and to post-mating behavioral changes. We discuss the reproductive tract musculature, various OA receptors, and interaction of OA signaling and male signals transferred during mating to communicate mating status. Finally, we conclude by proposing areas of future research on OA that remain to be explored. We focus the review on *D. melanogaster* as OA's functions in female reproduction are best characterized in this species. Additionally, we will discuss other insects whenever relevant and note when processes are conserved.

Structure, Innervation and OA receptors of the female reproductive tract

The typical insect female reproductive tract (RT) plan consists of two ovaries each composed of multiple egg-producing ovarioles. Ovarioles can be clustered together as in *D. melanogaster* (Miller 1950), or arranged evenly along the lateral oviducts as in stick insects and locusts (Fig 2A) (Lange 2009b). Germline stem cells reside in a niche at the anterior tip of the ovariole, and egg development progresses down the ovariole length. Mature oocytes are ovulated into lateral oviducts, which meet posteriorly to form a common oviduct.

In the uterus (also known as bursa or genital chamber) eggs are fertilized by sperm maintained in specialized sperm storage organs (SSOs). SSOs typically open into the uterus to optimize fertilization efficiency, but the number and morphology of SSOs varies across lineages. For example, *D. melanogaster* females have a tubular seminal receptacle (SR) and a pair of spermathecae (ST) (Bloch Qazi et al. 2003), while *L. migratoria* females have a single tubular spermatheca (Clark and Lange 2000). SSOs in some lineages have secretory capacity and provide substrates that maintain sperm viability and promote female fertility (Davey 1985; Lay et al. 1999; Lange and da Silva 2007; Schnakenberg et al. 2011; Sun and Spradling 2013). Fertilized eggs are then passed through the genital opening onto substrate (Klowden 2013). The female RT of some lineages have other specialized secretory organs collectively referred to as accessory glands. In *D. melanogaster*, these accessory glands are also known as parovaria.

Structures of the female RT are surrounded by visceral muscle. Like all insect muscles, visceral muscle is striated (Smith et al. 1966). However, unlike flight muscle, it is uninucleate, with more thin filaments per thick filament (Smith et al. 1966), fewer mitochondria, and a reduced sarcoplasmic reticulum (Schaefer et al. 1967; Klowden and Klowden 2010). Additionally, many visceral muscles display perforated z-discs characteristic of super contractile activity (Rice 1970; Nagai and Gordon Graham 1974; Middleton et al. 2006). Generally, each ovariole is surrounded by a muscular sheath (Cruickshank 1973; Akster and Smit 1977; Griffith and Lai-Fook 1986; Cook and Peterson 1989; Giorgi et al. 1990; dos Santos and Gregório 2002; Middleton et al. 2006; Sedra et al. 2015). In insects with clustered ovarioles, such as *D. melanogaster*, the ovarioles comprising each ovary are surrounded by an additional mesh-like layer of visceral muscle called the

peritoneal sheath (Cook and Meola 1978; Cook and Peterson 1989; Giorgi et al. 1990; Middleton et al. 2006; Sedra et al. 2015). The oviduct epithelium is also surrounded by muscle. In *D. melanogaster* the oviducts are surrounded by a single layer of circular muscles (Middleton et al. 2006), while in other insects the oviduct musculature consists of two perpendicular layers of circular and longitudinal muscle (Cook and Meola 1978; Thomas 1979; Cook et al. 1983; Robert et al. 1984; Sedra et al. 2015). In *D. melanogaster* the SR and spermathecal ducts contain a helically coiled layer of muscle cells (among other cell types and structures) (Nonidez 1920; Blaney 1970; Filosi and Perotti 1975), and in *L. migratoria* the ST is surrounded by both transverse and longitudinal muscle fibers (Lange and da Silva 2007). The contractility of RT musculature can be regulated by neurotransmitters and neuromodulators released from neurons innervating the reproductive tract.

The coordination of gamete movement with female mating status is made possible by neurons innervating the RT. OA neurons with cell bodies in the abdominal ganglion project to the reproductive tract through branches of the abdominal nerve trunk (Fig 2B) (Monastirioti 2003; Middleton et al. 2006; Rodríguez-Valentín et al. 2006; Rezával et al. 2014). They extensively innervate different regions of the RT and form type II neuromuscular junctions, which are typically associated with neurohormonal release (Middleton et al. 2006; Rodríguez-Valentín et al. 2006; Kapelnikov, Rivlin, et al. 2008; Atwood and Klose 2009; Avila et al. 2012). In *D. melanogaster*, approximately nine OA neurons that co-express the sex determination marker *doublesex* (*dsx*; henceforth $Tdc2^+$ dsx^+ neurons) constitute this population of OA neurons. It is worth nothing that these $Tdc2^+$ dsx^+ neurons are sexually dimorphic, with males having only three $Tdc2^+$ dsx^+ neurons (Rezával et al. 2014). Mating induces an increase in intracellular Ca^{2+} in $Tdc2^+$ dsx^+ cell bodies and increases the electrical activity of these neurons (Yoshinari et al. 2020). In *L. migratoria*, branches of the terminal (VIIIth) abdominal ganglion innervate different regions of the female RT, and some innervations are octopaminergic (Clark and Lange 2003).

Many studies have used quantitative or semi-quantitative mRNA amplification methods to assay OA receptor expression in various tissues. Many of OA's effects on egg development and sperm movement are thought to be mediated via receptors on the female reproductive tract. This role appears to be well conserved, as homologs of *D. melanogaster* OA receptors, especially OAMB (Octα1R) and Octβ2R, have been found in the female RTs of several insects (Table 1). In *D. melanogaster* OAMB and Octβ2R expression has been detected in the ovary, oviduct, SR, ST, parovaria and uterus (El-Kholy et al. 2015; Li et al. 2015). Reproductive tract expression of the other *Drosophila* OctβR homologs, Octβ1R and Octβ3R, are less consistent. Neither is highly expressed in the *D. melanogaster* female RT (El-Kholy et al. 2015; Li et al. 2015), but they can be found in the female RT of some other insects (Table 1). The presence of multiple OA receptors in different regions of the female RT indicates that OA has multiple functions within the RT. Additionally, OA receptors are commonly found in the insect CNS (Han et al. 1998; Verlinden et al. 2010; Sinakevitch et al. 2011; Lam et al. 2013; El-Kholy et al. 2015; H.-M. Li et al. 2016; Wu et al. 2017; Hana and Lange 2017b). OA receptors in the CNS may play a role in mediating reproductive behaviors (Zhou et al. 2012).

The wealth of genetic tools available in *D. melanogaster* has enabled more finely detailed characterization of OA receptor expression patterns at the cellular level in the female RT and CNS. Transgenic GAL4 lines driving UAS-GFP under control of *Oamb* regulatory elements reveal high *Oamb* expression within the oviduct epithelial layer, mature follicle cells in the ovary, and escort cells of the germarium (Lee et al. 2009; Deady and Sun 2015; Yoshinari et al. 2020). In the brain, *Oamb* is expressed in the mushroom bodies, the part of the brain controlling olfactory learning and memory, the insulin producing cells of the pars intercerebralis, as well as the optic lobes (Han et al. 1998; Crocker et al. 2010; Zhou et al. 2012; El-Kholy et al. 2015). Similar methods reveal high *Octβ2R* expression in the SR and lower portion of the uterus (El-Kholy et al. 2015; Li et al. 2015). Like *Oamb*, *Octβ2R* is also expressed in the mushroom bodies and pars intercerebralis (El-Kholy et al. 2015). *Octβ3R* and *Octβ1R* also show strong expression in the mushroom bodies, and in the antennal lobes and pars intercerebralis, respectively (El-Kholy et al. 2015). Analogous detailed characterization of OA receptor distribution in non-model insects will be useful for determining whether homologous OA receptors are modulating similar processes.

OA's role in female reproductive physiology

OA has multiple roles in insect female reproductive physiology. In insects, egg laying involves several steps (Bloch Qazi et al. 2003). First, viable mature oocytes must be produced in female ovaries. Next, mature oocytes must successfully exit the ovary and enter the oviducts during ovulation. Many studies in *Drosophila* have shown that OA plays a key role in modulating ovulation via follicle rupture (Deady and Sun 2015), and subsequent changes in female RT musculature contractility (Middleton et al. 2006; Rodríguez-Valentín et al. 2006; Rubinstein and Wolfner 2013). After ovulation, the oocyte, along with sperm stored in the SSOs, must then be able to enter the uterus to facilitate fertilization. Here, OA coordinates the movement of both gametes (Bloch Qazi et al. 2003; Avila et al. 2012). After fertilization, eggs are oviposited onto a substrate. Below, we detail OA's effects on each of the reproductive processes, and discuss known interactions of OA signaling with other molecules.

Ovulation

In *D. melanogaster*, both mated and virgin females are able to ovulate. *Tbh* mutant females are sterile due to defects in ovulation, causing accumulation of mature oocytes in their ovaries (Monastirioti et al. 1996; Monastirioti 2003). Sterility in these females can be rescued with OA feeding (Monastirioti et al. 1996; Cole et al. 2005). Additionally, simply injecting wild-type virgin females with OA is sufficient to induce ovulation (Meiselman et al. 2018). Interestingly, *Tdc2* mutant females lacking both OA and TA also exhibit egg retention, but not due to an ovulation defect (Cole et al. 2005). This suggests that TA may also play a role in egg movement. The OA required for ovulation comes from OA neurons innervating the reproductive tract. Silencing the female RT OA neurons causes egg retention (Rodríguez-Valentín et al. 2006), and expressing *Tbh* specifically in these neurons can rescue the sterility of *Tbh* mutant females (Monastirioti 2003).

OA exerts its effects on ovulation via at least two OA receptors expressed in the female RT. Mutations in the α-adrenergic receptor OAMB cause female sterility due to egg retention (Lee et al. 2003; Lee et al. 2009). Knockdown of *Oamb* specifically in stage 14 follicle cells reduces follicle rupture (Deady and Sun 2015), while expression of *Oamb* in the oviduct epithelium of *Oamb* mutant females rescues the sterility of these females (Lee et al. 2009). This indicates that OAMB has multiple functions in ovulation depending on its location. The β-adrenergic receptor Octβ2R is also needed for ovulation, as *Octβ2R* mutant females also exhibit the typical decreased egg laying due to an ovulation defect (Lim et al. 2014; Li et al. 2015).

It is possible that the role of OA in the egg laying process may be conserved. Injection of OA stimulates egg laying in the diamondback moth, *Plutella xylostella* (Li et al. 2020) and in the rice leaf bug, *Trigonotylus caelestialium* (Yamane 2013). In the hemipteran *Nilaparvata lugens*, knockdown of the β-adrenergic receptor NIOA2B2 results in the accumulation of eggs in ovaries (Wu et al. 2017). However, in the western tarnished plant bug, *Lygus hesperus*, and the malaria vector mosquito, *Anopheles gambiae*, injection of OA suppresses egg laying (Fuchs et al. 2014; Brent et al. 2016). In general, few studies in other insects have examined the reproductive fitness consequences of perturbed OA signaling, likely due to the lack of genetic tools.

During ovulation, OA modulates two independent, but synergistic pathways: follicle rupture and modulation of reproductive tract musculature. Additionally, OA signaling in the oviduct epithelium may also play a role in ovulation.

Follicle rupture—During ovulation, the layer of somatic epithelial follicle cells surrounding the oocyte is enzymatically degraded, releasing the mature oocyte. OA and follicular OAMB are required for this process (Deady and Sun 2015), as *Tbh* and *Tdc2* mutant females, as well as females with follicular knockdown of *Oamb*, exhibit reduced follicle rupture both *in vivo* and *in vitro* (Deady and Sun 2015). Follicle rupture competency is established in early to mid-stage 14, with the upregulation of the zinc finger transcription factor *hindsight (hnt)*, which is needed for the subsequent upregulation of *matrix metalloproteinase 2 (Mmp2)* and *Oamb* (Deady et al. 2017). OA binding to OAMB on follicle cells causes a rise in intracellular calcium, activating Mmp2 and initiating follicle rupture (Deady and Sun 2015). The source of the OA required for follicle rupture is not known, but is likely the OA neurons innervating the base of the ovaries. However, OA does not act alone to initiate follicle rupture; the steroid hormone 20-hydroxyecdysone (20E) is also required (Knapp and Sun 2017).

Muscle contraction—Follicle rupture alone does not promote successful ovulation, the oocyte must also be able to enter the oviducts, which requires changes in the contractility of RT musculature. Indeed, in *D. melanogaster*, virgin female oviducts have greater muscle tonus (Rubinstein and Wolfner 2013) and adopt a chiral loop conformation, which may prevent egg passage (Mattei et al. 2015). Muscle tonus decreases and the loop straightens after mating and the onset of egg laying (Rubinstein and Wolfner 2013; Mattei et al. 2015). Additionally, in *L. migratoria* and the stick insect, *Carausius morosus*, the contraction of the

oviduct induced by neural activation suppresses egg movements (Thomas 1979; Lange et al. 1984)

Ovary contraction.: Contraction of the ovarian muscles may be important for ovulation. In dipterans, *ex vivo* ovaries display spontaneous rhythmic contractions of the peritoneal sheath (Cook and Peterson 1989; Middleton et al. 2006; Meiselman et al. 2018). In *D. melanogaster* inhibiting ovary contraction by knocking down the NADPH oxidase Nox in muscle tissue causes egg retention, consistent with an ovulation defect (Ritsick et al. 2007). Also, contraction of *ex vivo* ovaries has been observed to drive eggs into the oviducts (Meiselman et al. 2018). Octopamine modulates the activity of the ovarian musculature (Table 2). In *D. melanogaster*, OA has been reported to increase both the amplitude (Middleton et al. 2006) and frequency (Meiselman et al. 2018) of ovary contractions. Similarly, in the stable fly, *Stomoxys calcitrans*, OA increases the amplitude of peritoneal sheath contractions (Cook and Peterson 1989), suggesting that OA modulation of ovary contraction may be important in other insects with clustered ovarioles. The OA receptor and downstream signaling pathways mediating OA's effect in the ovarian musculature remain unknown.

Inhibition of oviduct contraction.: OA also promotes ovulation by inhibiting oviduct muscle contraction. In *D. melanogaster* OA reduces the frequency and amplitude of oviduct contractions that are induced by neural activation (Rodríguez-Valentín et al. 2006). Additionally, activating *Tdc2* neurons to increase oviduct OA signaling is sufficient to decrease oviduct muscle tonus in a virgin female (Rubinstein and Wolfner 2013). OA's role in the oviduct musculature appears to be conserved, as OA is present in the female RT neurons and acts as an inhibitor of oviduct contraction in many insects (Table 2). However, in the cricket, *Gryllus bimaculatus*, OA application to the oviduct increases the frequency and amplitude of spontaneous oviduct contractions (Tamashiro and Yoshino 2014), suggesting that in some species other molecules may inhibit oviduct contraction.

Characterizing the signaling pathways downstream of OA in the musculature is essential to understanding how OA modulates ovulation. In *D. melanogaster* the OA receptor in the oviduct musculature mediating this relaxation is likely Octβ2R, as knockdown of this receptor in muscle tissue reduces the number of eggs laid by females, though this reduction is not as extreme as in an *Octβ2R* mutant (Li et al. 2015). Additionally, phentolamine, an inhibitor of OctβRs (Evans and Maqueira 2005), attenuates OA's effect on oviduct contraction in *L. migratoria* (Lange and Orchard 1986) and *Rhodnius prolixus* (Hana and Lange 2017a). Since OctβRs are found on the oviducts of many species (Table 1), their role in modulating oviduct contraction may be conserved. OctβRs are coupled to cAMP and, consistently, evidence in *D. melanogaster* suggests that OA acts in the oviducts via cAMP (Rodríguez-Valentín et al. 2006). OA's inhibition of oviduct contractions that are induced by neural activation is enhanced by the addition of the phosphodiesterase inhibitor IBMX (Rodríguez-Valentín et al. 2006). OA increasing cAMP in the oviduct is common to many species (Table 1), except in *Gryllus bimaculatus*, where OA has a contraction-stimulatory effect and acts via Ca^{2+} (Tamashiro and Yoshino 2014). Though the mechanism by which OA relaxes the oviduct is unknown in *D. melanogaster*, in *L.migratoria*, it has

been proposed that activation of protein kinase A downstream of cAMP may lead to phosphorylation of proteins resulting in muscle relaxation (Lange and Nykamp 1996). The pathways downstream of cAMP in oviduct muscle relaxation should be addressed in future studies.

It is intriguing to hypothesize that OA is promoting ovulation by simultaneously increasing contraction of the ovaries to force eggs out of the ovary, and inhibiting contraction of the oviducts to allow entrance of eggs, including into the lower RT. Recent advances in *in vivo* organ imaging (Koyama et al. 2020) may finally allow observation of the muscular events surrounding ovulation in intact females. OA's modulation of muscular activity, combined with its independent stimulation of follicle rupture, show that OA simultaneously coordinates multiple events required for ovulation.

The oviduct epithelium—In addition to its roles in follicle rupture, ovary contraction and oviduct muscle relaxation, in *D. melanogaster*, there may also be a role for OA in the oviduct epithelium. *Oamb* mutant sterility can be rescued by expressing *Oamb* in the epithelial layer of the oviduct (Lee et al. 2009). Unlike the situation in the musculature, OA signaling in the oviduct epithelium is relayed via Ca^{2+}, since constitutively active CaMKII expressed in the oviduct epithelium can also rescue *Oamb* mutant sterility (Lee et al. 2009). Additionally, applying OA to *ex vivo* oviducts elevates intracellular calcium in the epithelial tissue (Meiselman et al. 2018).

It has also been proposed that Octβ2R may also be required in the oviduct epithelium for ovulation (Lim et al. 2014). Expressing *Octβ2R* in the oviduct epithelium of *Octβ2R* mutant females using an *Oamb* promoter driven GAL4 rescues the Octβ2R ovulation defect. However, it has not been established through RNAi whether Octβ2R is necessary in this tissue for ovulation, thus it is difficult to interpret whether the rescue represents a restoration of endogenous function or a gain of function resulting from ectopic expression.

The role of OA in the oviduct epithelium and the epithelium's role in ovulation remain elusive, however it has been proposed that OA may serve to increase secretion into the oviduct lumen to aid in egg transport (Lee et al. 2009). Indeed, histology of the oviduct epithelium reveals the presence of microvilli, and cellular structures associated with secretory activity (Kapelnikov, Rivlin, et al. 2008). In addition, there is also evidence that OA secretion may increase after mating (Kapelnikov, Rivlin, et al. 2008). Secretory activity in the female reproductive tract has previously been linked to ovulation and egg deposition (Schnakenberg et al. 2011; Sun and Spradling 2013). However, further research is needed to fully elucidate OA's role in this tissue.

Integration of OA with other signals—OA is not the sole neuroactive molecule modulating ovulation. Glutamate, other biogenic amines, as well as various neuropeptides have been tested in a number of insects for their presence in the female RT and their effect on ovary and/or oviduct contraction (Table 2). Additionally, immunohistochemistry studies show that the distributions of OA, serotonin (5-HT), and the neuropeptide dromyosupressin within the female RT varies by tissue and region (Heifetz et al. 2014). Each tissue's unique complement of neuroactive molecules may help give rise to each tissue's characteristic

function (Heifetz et al. 2014). For instance, *Tdc2*$^{+}$ *dsx*$^{+}$ neurons are both octopaminergic and glutamatergic, and it has been proposed that OA can modulate stimulatory glutamatergic signaling in the oviduct (Rodríguez-Valentín et al. 2006). However, it is as yet unknown whether OA and glutamate are released simultaneously *in vivo*. Similarly, in *L. migratoria* OA and the stimulatory neuropeptide proctolin are found to have antagonistic effects. OA is found to inhibit proctolin induced contractions while proctolin attenuates OA's stimulation of cAMP (Nykamp and Lange 2000). In general, the relationship between OA and these other signaling molecules in regards to ovulation is unknown, and presents an intriguing avenue for future research.

OA signaling in the female RT is also regulated by internal endocrine signals. In this way, OA signaling and downstream ovulation processes can be adjusted in accordance with the female's internal state. One such signal is the peptide ecdysis triggering hormone (ETH). ETH released from adult female inka cells binds to ETH receptor (ETHR) on *Tdc2* neurons innervating the female RT, resulting in OA release and stimulation of ovary contraction (Meiselman et al. 2018). Females unable to release ETH, and those lacking ETHR in *Tdc2* neurons exhibit egg retention characteristic of an ovulation defect (Meiselman et al. 2018).

When females are exposed to stressful conditions, such as heat stress or starvation, fecundity declines, as females must allocate resources to somatic maintenance over reproduction (Dillon et al. 2009; Marshall and Sinclair 2010; Meiselman et al. 2018). One way that females suppress egg laying during heat stress is by reducing ovulation. High levels of 20E brought on by increasing temperatures (Hirashima et al. 2000; Gruntenko et al. 2003) inhibits ETH release from inka cells (Meiselman et al. 2018), reducing OA signaling in the female RT. Treating heat stressed females with ETH restores ovulation.

Nutrition and Metabolism

OA modulates female physiology and behavior during starvation (Yang et al. 2015; Corrales-Carvajal et al. 2016; Yu et al. 2016; Damrau et al. 2017; Tian and Wang 2018; Sayin et al. 2019; Selcho and Pauls 2019; Roeder 2020) to help maintain energy homeostasis. OA may also modulate the shift in energy balance that occurs with the energetically expensive rise in egg production after mating. One way OA may affect female metabolism is through insulin signaling. Both OA neurons and OA receptors *Oamb, Octβ2R, and Octβ1R* are found in the insulin producing region of the brain (Crocker et al. 2010; Luo et al. 2014). *Tbh* mutants, *Octβ2R* and *Octβ1R* all show changes in release of the insulin-like peptide dILP2 from insulin producing cells (Y. Li et al. 2016; Li et al. 2017). Knockdown of *Octβ2R* and *Octβ1R* within the energy-storing fat body also affect dILP2 levels in the brain, (Li et al. 2017), suggesting that OA regulation of insulin signaling is complex, possibly involving multiple tissues. OA modulation of insulin signaling could also have reproductive consequences, as dILPs are known to regulate germline stem cell division (LaFever and Drummond-Barbosa 2005; Hsu and Drummond-Barbosa 2009).

OA also affects female metabolism through energy storage and mobilization. *Tbh*, *Octβ2R*, and *Octβ1R* mutant females exhibit high triglyceride levels (Y. Li et al. 2016; Li et al. 2017), while *Oamb* mutants had reduced triglyceride levels relative to controls (Erion et al. 2012; Li et al. 2017). Activation of OA neurons using a *Tdc2-GAL4* driver increased triglyceride

levels (Erion et al. 2012), while activating OA neurons with *Tbh-GAL4* produced the opposite effect (Y. Li et al. 2016). These conflicting results could be due to disparate effects of OA and TA on lipid levels. In addition to *Drosophila*, OA has been shown to affect energy mobilization in a variety of other insects (Orchard et al. 1982; Fields and Woodring 1991; Park and Keeley 1998; Meyer-Fernandes et al. 2000; Corby-Harris et al. 2020). In *Drosophila*, OA's effect on lipid storage could be a consequence of its effect on insulin signaling, as Erion (2012) found that dILP mutants suppressed the rise in triglyceride level caused by activating OA neurons. Insulin signaling has previously been shown to regulate fat body lipid metabolism (DiAngelo and Birnbaum 2009). OA regulation of the fat body may have important reproductive consequences as the fat body is also the main site of yolk protein synthesis (Hames and Bownes 1978). Given the links between OA, insulin signaling, and energy storage, it is possible that post-mating changes in OA signaling could modulate female metabolism to cope with the energetic demands of egg production. Deciphering OA's role in mated female metabolism should be addressed by future research.

Changes in OA signaling are essential for the female post-mating responses

Reproduction-related OA signaling needs to be tightly controlled and sensitive to signals that indicate the transition from the virgin to the mated state. During mating, females receive sperm and a cocktail of seminal fluid proteins (Sfps; proteins secreted by the male accessory glands, ejaculatory bulb, ejaculatory duct, and seminal vesicles). Though OA is essential for adult female reproductive physiology in unmated females regardless of age, it plays an even more crucial role in interacting with sperm and Sfps and regulating aspects of the female post-mating response (PMR). The PMR comprises a suite of physiological and behavioral changes that switch the female from a non-reproductive virgin state to a fecund mated state (Avila et al. 2011).

OA's role in enhancing post-mating egg laying above basal, virgin levels has been extensively studied. OA increases germline stem cell number (Yoshinari et al. 2020) and ovulation after mating. Sperm also become available after mating, and OA regulates the release of sperm from SSOs for fertilization. In addition to physiological changes, mated females undergo behavioral changes to optimize their reproductive output: they increase food intake and reduce sexual receptivity. The interaction of OA signaling with male signals (Sfps) helps to further couple the energetically costly PMR to the female's mating status. Below, we detail OA's effects on each of the reproductive processes, and discuss known interactions of OA signaling with Sfps and other molecules.

It is worth noting that the intensity and duration of PMR can be variable and depends on male and female genotypes (Chow et al. 2010; Delbare et al. 2017). Females and males typically diverge in their reproductive strategies, and hence their ideal remating interval and reproductive output. Sfps and their female signaling partners function at the interface of sexual conflict and post-mating sexual selection, mediating a molecular 'arms race' while simultaneously ensuring the reproductive success of both sexes.

Regulation of OA signaling post-mating

One way in which OA signaling may be modulated post-mating is through transcriptional regulation of OA pathway components and receptors. Increasing transcription of one or more OA pathway components could enhance OA signaling, and lead to PMR phenotypes. Nonetheless, in general, transcriptomic studies have failed to find consistent mating-induced changes in OA related gene expression. Many transcriptome studies in *D. melanogaster* (Lawniczak and Begun 2004; McGraw et al. 2004; Mack et al. 2006; Kapelnikov, Zelinger, et al. 2008; McGraw et al. 2008; Innocenti and Morrow 2009; Prokupek et al. 2009; Dalton et al. 2010; Parisi et al. 2010; Gioti et al. 2012; Short and Lazzaro 2013; Zhou et al. 2014; Delbare et al. 2017; Fowler et al. 2019; Newell et al. 2020) and other insects (Bono et al. 2011; Gomulski et al. 2012; Gabrieli et al. 2014; Manfredini et al. 2015; Alfonso-Parra et al. 2016; Al-Wathiqui et al. 2016; Alonso et al. 2019; Liu and Hao 2019; Gao et al. 2020; Pascini et al. 2020) have shown that mating triggers extensive female transcriptional changes. Although in *D. melanogaster*, comparing these studies is difficult due to the differences in methodologies, timepoints, strains, and tissues used (for instance, post mating up/down-regulation of *Tbh* can vary by female genotype), no study to date has identified an enrichment of gene ontology (GO) terms associated with OA. However, the level of whole-body *Tbh* expression at 5-6 hr post-mating is correlated with female egg production on the first day after mating (Delbare et al. 2017), suggesting that mating can change in OA-related gene expression.

Given the pleiotropic nature of OA's role in reproduction and other non-reproductive processes, tissue-specific studies might be more informative for assessing post-mating transcriptional regulation of OA in reproduction. In the *D. melanogaster* SR, Prokupek (Prokupek et al. 2009) found downregulation of *Octβ2R* and *Oamb* at both 3 and 6 hrs. In contrast, neither Mack (Mack et al. 2006) nor Kapelnikov (Kapelnikov, Zelinger, et al. 2008) found mating-induced transcriptional regulation of OA-related genes in the lower female RT or the oviduct, respectively. These results indicate that response to OA in various regions of the female RT may be differentially regulated. It is also likely that mating-induced changes in OA signaling are regulated through post-transcriptional means, such as vesicle release (Heifetz and Wolfner 2004).

Generally, transcriptomic studies in other insects have also failed to find consistent mating-induced differential expression of OA-related genes (Bono et al. 2011; Gomulski et al. 2012; Gabrieli et al. 2014; Manfredini et al. 2015; Alfonso-Parra et al. 2016; Liu and Hao 2019). Two isolated exceptions are found in the burying beetle, *Nicrophorus vespilloides*, where *Octβ2R* and *Octβ1R* are upregulated 3 days after mating (Cunningham et al. 2014), and in the tobacco cutworm moth, *Spodoptera litura*, where OAMB is upregulated 1 day after mating (Gao et al. 2020).

Current evidence suggests that OA's role in the PMR is regulated by increased activity of OA neurons within the female RT. This increase in OA neuronal activity has been shown in several ways. After mating there is an increase in the number of type II neuromuscular junctions innervating the oviduct musculature (Kapelnikov, Rivlin, et al. 2008; Rubinstein and Wolfner 2013). Such synaptic outgrowth is generally in response to elevated synaptic activity (Budnik et al. 1990). Additionally, genetically encoded Ca^{2+} indicators, which

report levels of neuronal activity, show a subset of OA neurons that innervate the female RT (likely the *Tdc2*$^{+}$ *dsx*$^{+}$ neurons) are more active after mating (Yoshinari et al. 2020). Immunohistochemistry studies also show that the intensity of OA staining at female RT nerve terminals decreases at various locations and time points after mating, indicative of OA release (Heifetz et al. 2014). Subsequent higher levels of OA release within the female RT after mating may facilitate elevated oogenesis, ovulation, sperm release, and other OA-associated aspects of the PMR.

Post-mating changes in OA neuronal activity can be influenced by ejaculate components. The sensitivity and response of OA signaling to male signals is best studied in *D. melanogaster*, where immunohistochemistry has revealed extensive mating-induced changes in OA release. Virgin females have low OA release from nerve termini in the reproductive tract, but ejaculate components received during mating promote OA release (Heifetz et al. 2014). At 20 minutes post-mating, substances from the male accessory gland are needed to stimulate OA release in the ovary and inhibit OA release in the uterus (Heifetz et al. 2014). Changes in OA immunofluorescence in the lateral oviduct at 90 minutes post-mating requires sperm, while changes in OA immunofluorescence at 180 minutes post-mating in the common oviduct and uterus require only the experience of mating (Heifetz et al. 2014). One Sfp, ovulin, has been linked to enhanced OA neuronal signaling and OA neuron outgrowth in the lateral oviducts (Rubinstein and Wolfner 2013).

To date, no specific male proteins have been shown to modulate female OA signaling in other insects. However, in *L. migratoria* a male derived myotropin transferred to the female (Paemen et al. 1990) can stimulate oviduct contraction *ex vivo* (Paemen et al. 1991). In the stable fly *Stomoxys calcitrans*, extracts from male reproductive tracts can also modulate oviduct muscle activity (Cook and Wagner 1992). In addition, male accessory gland products from *Aedes aegypti*, *L. migratoria*, *Helicoverpa armigera*, and *Adalia bipunctata* have been shown to stimulate oviposition (Leahy and Craig 1965; Lange and Loughton 1985; Jin and Gong 2001; Perry and Rowe 2008). It is possible that these male proteins from other species may be modulating female OA signaling as well.

In *D. melanogaster*, mating dynamically influences the neuronal release of OA and other neuroactive molecules within the female RT (Heifetz et al. 2014). Soon after mating 5-HT immunoreactivity decreases in the ovary and SR, while dromyosuppressin immunoreactivity decreases in the oviduct, suggesting that these compounds are being released. Changes in the pattern of OA release were not detected immediately after mating. Later, at 90 minutes post-mating, 5-HT immunoreactivity increases in the common oviduct, suggesting it is accumulating at nerve terminals. At the same timepoint, dromyosuppressin immunoreactivity decreases in the ovary, while OA immunoreactivity decreases in the SR and uterus (Heifetz et al. 2014). The unique combinations of neuroactive compounds present in each female RT tissue, coupled with tissue-specific mating-induced changes in signaling molecule release may facilitate the unique PMRs seen in different female RT regions. Additionally, how OA signaling integrates with other signaling molecules to coordinate reproductive events is not yet understood, and presents a promising avenue for future research. We next discuss the involvement of OA in PMRs in each of the female RT regions.

Oogenesis and the female's post-mating response

Egg production begins with the asymmetric division of germline stem cells (GSCs) within the germarium. In *D. melanogaster* this process is tightly controlled by somatic cells within the niche (cap cells, escort cells, terminal filament cells), as well as by endocrine signals (Ameku and Niwa 2016; Ameku et al. 2018). After mating, the average number of GSCs increases. This increase is the result of elevated activity of $Tdc2^{+}$ dsx^{+} OA neurons innervating the ovary (Yoshinari et al. 2020). Activation of these neurons is sufficient to produce a GSC increase in virgin females (Yoshinari et al. 2020).

OA binding to OAMB on escort cells activates Mmp2, via a rise in intracellular Ca^{2+} (Yoshinari et al. 2020). Activated escort cell Mmp2 then stimulates GSC proliferation via an unknown mechanism (Yoshinari et al. 2020). However, OA is not the sole signaling molecule regulating post-mating GSC proliferation; ovarian produced 20E, as well as midgut derived neuropeptide F are also required, but how they interact with OA signaling in escort cells is not yet understood (Ameku and Niwa 2016; Ameku et al. 2018). The rise in GSC number after mating is important for optimal female fertility, as abolishing the post-mating GSC increase by knocking down the 20E synthesis pathway gene *neverland* in the germaria somatic cells significantly decreases female fertility (Ameku and Niwa 2016). The involvement of OA, OAMB, and Mmp2 in both GSC proliferation and follicle rupture suggests that common regulatory pathways are acting at different stages of germline development. In this way, OA may serve to help sustain increased post-mating egg production by coordinating egg loss from the ovary with GSC proliferation and the development of new eggs.

The rise in OA signaling required for the post-mating increase in GSC number might potentially be connected to the male Sfp Sex Peptide (SP), via binding to its receptor in females, the Sex Peptide Receptor (SPR) (Yapici et al. 2008). SP is a 36-amino-acid peptide (Chen et al. 1988), and is one of the most well-characterized Sfps in *Drosophila*. SPR is a GPCR expressed in SP sensory neurons (SPSNs), whose cell bodies are located on the uterus and arborize within the uterine lumen and innervate the common oviduct (Yapici et al. 2008; Häsemeyer et al. 2009; Yang et al. 2009). SPSNs project to the tip of the abdominal ganglion, where they are presynaptic to and physically interact with $Tdc2^{+}$ dsx^{+} neurons through inhibitory cholinergic signaling (Yoshinari et al. 2020). Mating silences SPSNs, and thereby activates $Tdc2^{+}$ dsx^{+} neurons (Yoshinari et al. 2020). We refer to the process by which SP-SPR binding silences the SPSNs, and consequently activates the $Tdc2^{+}$ dsx^{+} neurons as the SP-SPSN-OA axis.

Ovulation and the female's post-mating response

The post-mating rise in ovulation is partly spurred by increased OA signaling in the oviduct relaxing oviduct musculature. Whether increasing OA neuronal activity after mating enhances follicle rupture and ovary contraction has not yet been explored. OA release from lateral oviduct nerve terminals increases at 180 minutes post-mating, coinciding with the onset of elevated ovulation (Heifetz et al. 2014) and changes in oviduct conformation (Mattei et al. 2015). The male accessory gland protein, ovulin, has been shown to modulate OA signaling in the female reproductive tract after mating to promote elevation of ovulation

in the first 24 hours post-mating (Herndon and Wolfner 1995; Heifetz et al. 2000; Rubinstein and Wolfner 2013). Females mated to ovulin null mutant males exhibit reduced ovulation and higher oviduct muscle tonus (Heifetz et al. 2000; Rubinstein and Wolfner 2013). Ovulin's effects on ovulation can be the result of increasing the female's sensitivity to OA. However, it is more likely that ovulin increases the activity of OA neurons, as ovulin stimulates an increase in number of synaptic boutons from OA neurons onto the oviduct musculature after mating (Rubinstein and Wolfner 2013). How ovulin increases OA signaling is not known, but since ~10% of transferred ovulin enters the hemolymph after mating (Lung and Wolfner 1999), ovulin could be acting on local targets in the female RT, the CNS, or both. Nonetheless, since the lack of ovulin does not fully abolish ovulation, additional pathways must also contribute to increasing OA signaling and ovulation after mating.

Sperm storage

Females of many insect species store sperm for an extended period of time, from several days to years, or even decades in some hymenoptera species (Neubaum and Wolfner 1999). Sperm storage temporally uncouples mating and progeny production, enabling extended fertility and efficient gamete usage. In the context of multiple mating, storage of various males' sperm also promotes sperm competition and selective sperm use. Sperm storage consists of three stages: accumulation of sperm into SSOs, maintenance of sperm within, and the regulated release of sperm from SSOs. To achieve optimal reproductive output and avoid gamete wastage, the timing of ovulation must be coordinated with sperm release. Among insects, the involvement of OA signaling in sperm storage has been demonstrated in *D. melanogaster* and *L. migratoria*.

In *D. melanogaster*, the SR and ST are structurally and functionally distinct, with SR acting as the main SSO. Virgin females accumulate OA at nerve termini innervating the SR; by 90 minutes after mating, OA is released from these nerve termini to surrounding tissues and this release continues until at least 180 minutes after mating (Heifetz et al. 2014). OA and other signaling molecules' production and release are controlled dynamically after mating to exert combinatorial effects on sperm storage and other post-mating events (Heifetz et al. 2014).

By studying OA- and TA-less *Tdc2* mutants and OA-less *Tbh* mutants, Avila et al. (2012) showed that although OA and TA are not required for sperm accumulation into storage, OA is required for efficient sperm release from the SR, and both neuromodulators are required for efficient sperm release from SR and ST (Avila et al. 2012). Loss of Tbh or Tdc2 results in sperm retention (Avila et al. 2012). It is worth noting that these sperm retention defects do not depend directly on ovulation, because a different egg-retention mutant (*logjam*) releases sperm faster than control females (Avila et al. 2012). *Drosophila* SSOs do not have sphincters at their openings (Filosi and Perotti 1975), suggesting that OA neurons might regulate sperm release not by precise control of sperm movements, but by modulating the musculature of SSOs, spermathecal duct rotations, production of secretions to promote sperm motility, or by regulating fluid movements (Bloch Qazi et al. 2003; Middleton et al. 2006; Schnakenberg et al. 2011; Sun and Spradling 2013).

The OA receptor OAMB is expressed in the parovaria and might act remotely to mediate sperm release from storage (Avila et al. 2012). The function of parovaria remains largely elusive, but their secretory roles suggest that OAMB might regulate sperm release by stimulating secretion in the parovaria, similar to its role in the oviduct epithelium. Future studies should also investigate the involvement of *Octβ2R*, which is expressed in the SSOs (El-Kholy et al. 2015; Li et al. 2015), in sperm storage.

Sperm storage also enables sperm competition and female sperm preference. In a multiple mating context, OA neuron-specific knockdown of each of three genes (*caup*, *hid*, and *Rab2*) in a female affects the relative paternity success of her mates (Chen et al. 2019). Because each of these genes serves developmental or neuronal functions, their knockdown likely impacts OA neuron function, which in turn affects competitive sperm usage. Sperm competition is complex and involves multiple steps after both matings (Manier et al. 2010). Given OA's involvement in facilitating efficient sperm release, perturbing OA neuron function might result in the abnormal retention of any male's sperm. Future research is needed to elucidate the precise mechanism by which OA signaling exerts its effect on sperm competition.

In *L. migratoria*, OA-immunoreactive neurons with cell bodies in the VIIth and VIIIth (terminal) abdominal ganglia project to the female RT and send innervations to the ST (Clark and Lange 2000; Clark and Lange 2003; Lange and da Silva 2007). In *ex vivo* ST preparations, OA stimulates spermathecal myogenic and neurogenic contractions in a dose-dependent manner likely through cAMP, and phentolamine and schistoFLRFamide inhibits OA-induced spermathecal contractions (Clark and Lange 2003). Interestingly, OA's effect on spermathecal muscles is opposite to that on the oviduct: it relaxes oviduct muscles while promoting the contraction of ST. As such, the same neuromodulator can efficiently facilitate ovulation and sperm release simultaneously. OA's constraction-stimulating effect is also similar to its effect on promoting peritoneal sheath contraction in the ovaries of *D. melanogaster*. Finally, similar to ovulation, a few additional neuropeptides and biogenic amines including TA, 5-HT, proctolin and crustacean cardioactive peptide (CCAP) are involved in modulating sperm storage in *L. migratoria* and other insects (Table 2).

Behavior

In female insects, the PMR also includes behavioral responses. One of the best characterized behavioral PMR is the change in sexual receptivity: virgin females are typically receptive, and after mating they become unreceptive (or much less receptive) to male courtship and mating attempts. In *D. melanogaster*, $Tdc2^{+}$ dsx^{+} neurons facilitate this PMR (Rezával et al. 2014). Thermogenetically activating $Tdc2^{+}$ dsx^{+} neurons in virgin females reduces their receptivity, while silencing $Tdc2^{+}$ dsx^{+} neurons in mated females abolishes PMR and increases their receptivity to remating (Rezával et al. 2014). It is worth noting that despite being regulated by the same $Tdc2^{+}$ dsx^{+} neurons, receptivity can be genetically uncoupled from egg production (Barnes et al. 2007). Indeed, an enhancer-GAL4 line (VT7068) labels a subset of OA-like neurons that, when silenced, reduces a virgin female's receptivity with no effect on egg laying (Feng et al. 2014). It will be intriguing to determine if subsets of OA neurons interact with different neuronal circuits and facilitate different aspects of

PMR. In particular, with the female egg laying circuit recently mapped out (Wang et al. 2020), it opens the opportunity to explore OA's involvement in this circuit, and how it may interact with a receptivity circuit. Among male-derived signals, SP has been shown to be particularly important for reducing mated females' receptivity. However, while SPSNs physically interact with *Tdc2*$^{+}$ *dsx*$^{+}$ neurons to promote oogenesis, it is as yet unknown if receptivity is regulated by the same SP-SPSN-OA axis.

In *D. melanogaster* other PMRs include changes in sleep, feeding, activity, memory, aggression, immune response and longevity (Reviewed in (Avila et al. 2011); see also (Scheunemann et al. 2019)), and OA signaling has been linked to a few of them. To accommodate the energetic demands of egg production, females increase starvation-induced food seeking (Yu et al. 2016) or protein deficiency-induced nutrient seeking after mating (Tian and Wang 2018), and OA signaling is required for an increase in yeast appetite but not salt appetite (Walker et al. 2015). Furthermore, OA is involved in regulating reproduction in response to infection (Kurz et al. 2017; Masuzzo et al. 2019). A subset of brain OA neurons that express an NF-κB pathway component are activated by bacterial peptidoglycan and reduce egg laying by inhibiting follicle rupture (Kurz et al. 2017). This OA neuron subset is not the *Tdc2*$^{+}$ *dsx*$^{+}$ neurons mentioned above, and it is unknown how it regulates follicle rupture (Masuzzo et al. 2019). Females also become more aggressive after mating (Ueda and Kidokoro 2002; Nilsen et al. 2004; Bath et al. 2017), fighting for more than twice as long as virgin females do (Bath et al. 2017). Although OA's role in regulating male aggression has been studied extensively (Andrews et al. 2014; Watanabe et al. 2017; Rillich et al. 2019), its role in female aggression is yet to be elucidated. Future studies can dissect OA's involvement in these PMR at the neuronal circuit level, and how they are modulated by mating status.

Although behavioral PMR has not been investigated in other insects to the level of molecular and neuronal detail as in *D. melanogaster*, OA's involvement is commonly observed. In *L. migratoria*, OA modulates the coordination of digging of the oviposition hole and oviposition of fertilized eggs (Wong and Lange 2014). In the subsocial burying beetle *Nicrophorus vespilloides*, OA receptors are differentially expressed by mating and social conditions (Cunningham et al. 2014). Finally, in the adzuki bean beetle, *Callosobruchus chinensis*, injection of OA reduces receptivity, but genetic variation across natural populations are associated with different sensitivities to OA and intensities of PMRs (Yamane and Miyatake 2010; Yamane 2014). These results suggest potentially conserved roles of OA in behavioral PMR across insect lineages.

Conclusions

In this review, we have discussed how OA helps the female transition from a virgin to a mated state, and coordinates multiple aspects of female post-mating physiology and behavior. For example, in *D. melanogaster* mating increases the number of type II neuromuscular junctions in OA neurons of the female RT, thereby increasing OA release. OA then binds to OA receptors throughout the female RT to increase GSC number, promote ovulation (via follicle rupture and inhibition of oviduct contraction), and facilitate sperm release from storage. OA is also involved in effecting post-mating behavioral changes.

Additionally, OA signaling is integrated with other neuronal and endocrine signals of the female internal state to optimize reproductive output. Expression of different OA receptors and presence of other signaling molecules enable region-specific responses to realize different outcomes.

Given OA's importance to female fertility, it is interesting that male Sfps have evolved to enhance female reproduction by manipulating female OA signaling. Furthermore, OA's modulation of reproductive processes, particularly oviduct contractility, is well-conserved throughout insects, suggesting OA is critical throughout the evolution of insect reproduction. We conclude by proposing some areas of future exploration that can further expand our appreciation of OA's pivotal role in reproduction.

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Abbreviations

20E	20-hydroxyecdysone
5-HT	5-hydroxytryptamine (serotonin)
Ca^{2+}	calcium
cAMP	adenosine monophosphate
CNS	central nervous system
GPCR	G protein-coupled receptor
GSC	germline stem cell
ETH	ecdysis triggering hormone
ETHR	ecdysis triggering hormone receptor
OA	octopamine
PMR	post-mating response
RT	reproductive tract
Sfp	seminal fluid protein
SP	Sex Peptide
SPR	Sex peptide receptor
SPSN	SP sensory neurons

SR	seminal receptacle
SSO	sperm storage organ
ST	spermatheca(e)
TA	tyramine
VNC	ventral nerve cord

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Questions for future exploration

- How does OA coordinate different reproductive events at the neural circuit level? In *D. melanogaster*, does each $Tdc2^{+}$ dsx^{+} neuron subtype innervate a different region of the RT and have a distinct function? One way to approach this question could be mosaic labeling and manipulation of each $Tdc2^{+}$ dsx^{+} neuron and careful characterization of reproductive phenotypes to find correlations between individual $Tdc2^{+}$ dsx^{+} neurons, OA levels, and reproductive outcomes.
- How does OA interact with other signaling molecules present in the female RT (e.g. TA, glutamate, 5-HT, dopamine, and various peptides) to confer region-specific identity and exert combinatorial control? Characterizing the spatiotemporal expression dynamics of each of these molecules in wildtype and mutant backgrounds might be a starting point.
- Is there natural variation in OA signaling, and if so, does it correlate with variation in PMR strength? Such variation might manifest in OA titer and metabolism, numbers, innervation and connectivity of OA neurons, response of OA to physiological events (e.g. mating), and sequence and expression of OA receptors.

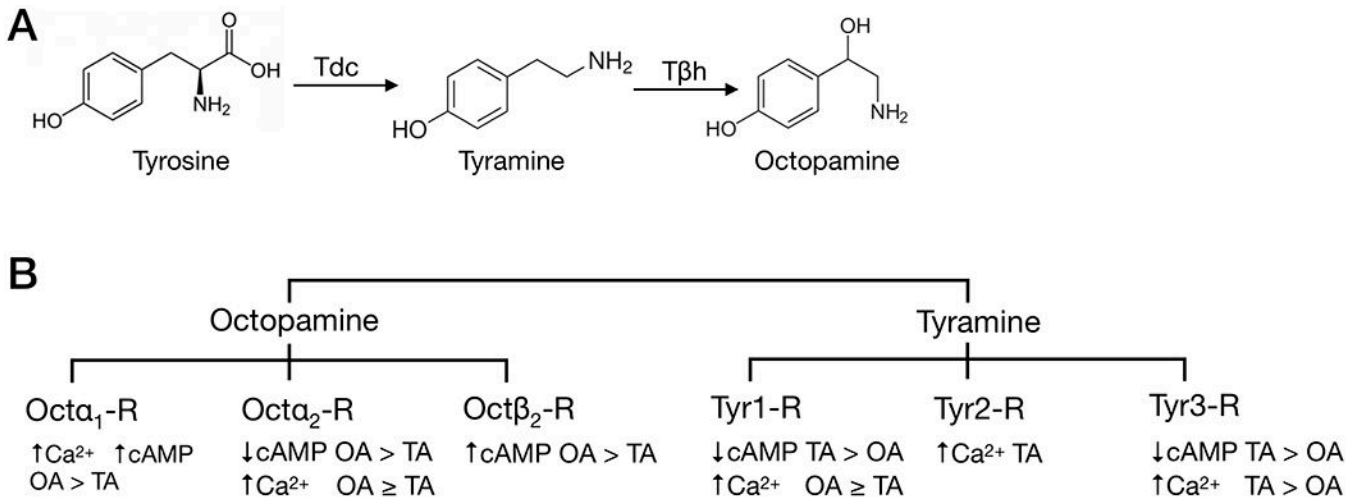

Figure 1.

Octopamine biosynthetic pathway and classification of octopamine receptors. **A** Tyrosine decarboxylase (TDC) converts tyrosine to tyramine by decarboxylation. Tyramine is hydroxylated by tyramine β-hydroxylase (TBH) to produce octopamine. **B** Classification of octopamine receptors based on schemes from Evans and Maqueira (2005), Farooqui (2012), Wu (2014), and Hana and Lange (2017). OA=octopamine, TA=Tyramine, cAMP=cyclic adenosine monophosphate, Ca^{2+}=Calcium.

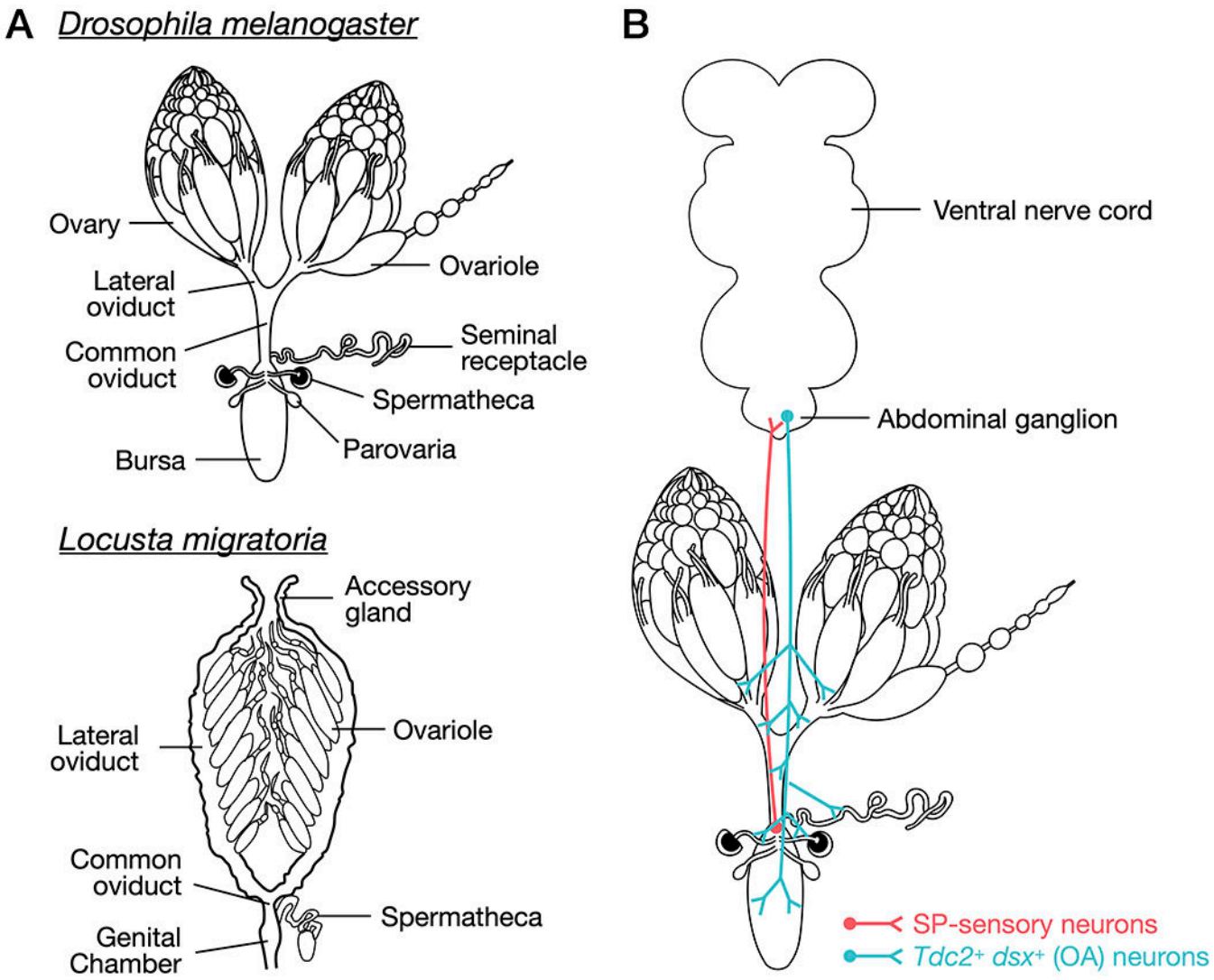

Figure 2.

Features of the insect female reproductive tract. **A** Diagram of the *Drosophila melanogaster* female reproductive tract (top) and the *Locusta migratoria* female reproductive tract (bottom). *L. migratoria* diagram based on (Lange 2009b). *D. melanogaster* diagram based on (Miller 1950). **B** Locations of *Tdc2*$^{+}$ *dsx*$^{+}$ neurons and SPSNs in the *D. melanogaster* female reproductive tract and VNC. Circle depicts cell bodies and ‘v’ shapes depict innervations.

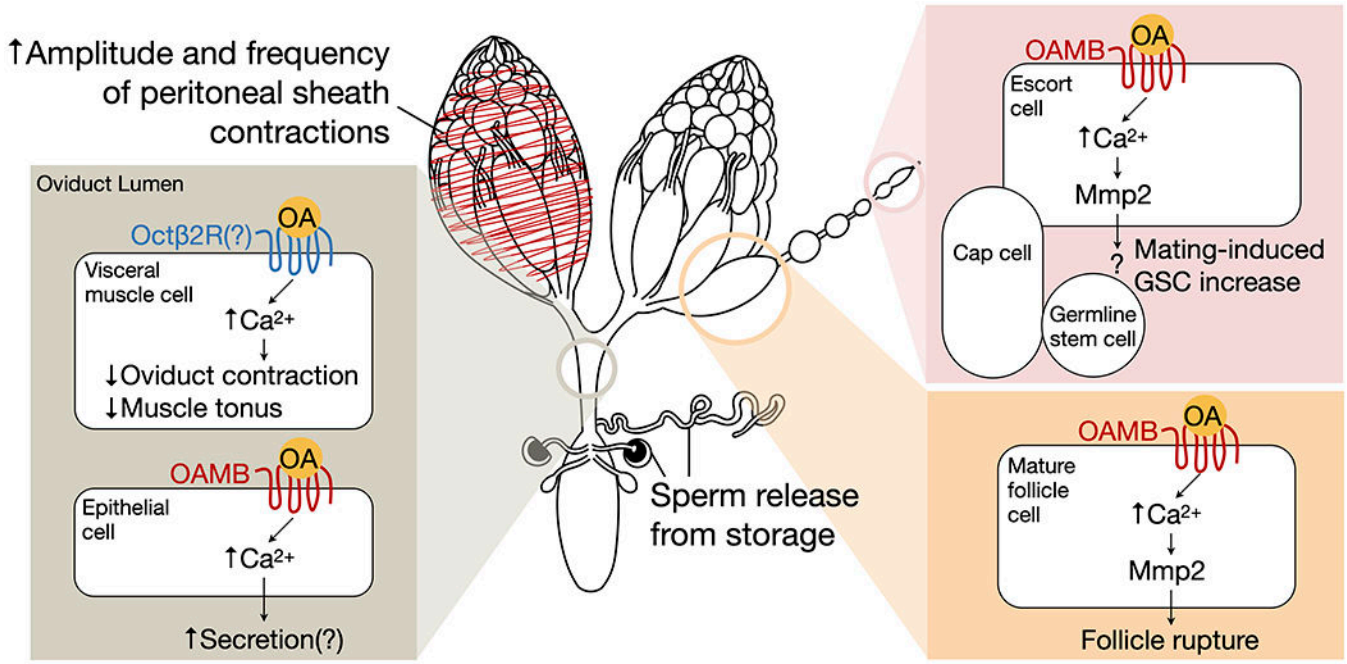

Figure 3.

Summary of octopamine functions within the *Drosophila melanogaster* female reproductive tract. Red lines on the ovary represent the peritoneal sheath musculature. Colored boxes on the sides illustrate signaling pathways in specific regions of the reproductive tract.

Table 1

Octopamine (OA) receptors are expressed in female reproductive tract tissues of many insect species. A dot indicates an OA receptor gene is expressed in the specified tissue, a line indicates an OA receptor gene has low or no expression in the specified tissue, and gray indicates the expression pattern has not been tested.

Species (*Order*)	Receptor	Ovary	Oviduct	Uterus	Spermatheca	Other	Source
Drosophila melanogaster (*Diptera*)	Oamb	•	•	•	•	Seminal receptacle • Parovaria •	(El-Kholy et al. 2015; Li et al. 2015)
	Octβ1R	-	-	-	-	Seminal Receptacle - Parovaria -	(El-Kholy et al. 2015; Li et al. 2015; Leader et al. 2018)
	Octβ2R	•	•	•	•	Seminal receptacle • Parovaria •	(El-Kholy et al. 2015; Li et al. 2015)
	Octβ3R	-	-	-	-	Seminal Receptacle - Parovaria -	(El-Kholy et al. 2015)
	Octα2R	-			-		(Leader et al. 2018)
Trichoplusia ni (Lepidoptera)	Octα1R		•				(Lam et al. 2013)
	Octβ1R		•				
	Octβ2R		•				
	Octβ3R		-				
Pieris rapae (Lepidoptera)	Octα1R		•				(Lam et al. 2013)
	Octβ1R		•				
	Octβ2R		•				
	Octβ3R		•				
Nicrophorus vespilloides (Coleoptera)	Octα1R		•				(Cunningham et al. 2014)
	Octβ1R		-				
	Octβ2R		•				
	Octβ3R		-				
Apis melifera (Hymenoptera)	Octα1R	•					(Vergoz et al. 2012)
Rhodnius prolixus (Hemiptera)	Octβ2R	•	•	•		Cement gland •	(Hana and Lange 2017b)
Nilaparvata lugens (Hemiptera)	Octβ2R	-	•		•	Copulatory pouch •	(Wu et al. 2017)
Pseudaletia unipuncta (Lepidoptera)	Octα1R		-				(Lam et al. 2013)
	Octβ1R		-				
	Octβ2R		-				
	Octβ3R		-				
Bactrocera dorsalis (Diptera)	Octβ1R	-					(H.-M. Li et al. 2016)

Legend: Not tested • Expressed - Low/no expression

Table 2

Neuroactive molecules and their effects on contractility of muscles in the female reproductive tract.

Species (Order)	Confirmed Present in female RT	Neuroactive Molecule	Effect on Oviduct contraction	Secondary Messenger	Effect on Ovary contraction	Secondary Messenger	Effect on SSO	Secondary Messenger	Source
***Drosophila melanogaster* (Diptera)**	Y	Glutamate	Stimulatory		No Effect				(Rodríguez-Valentín et al. 2006; Meiselman et al. 2018)
	Y	OA	Inhibitory	cAMP	Stimulatory		Promote sperm release *		(Middleton et al. 2006; Rodríguez-Valentín et al. 2006; Avila et al. 2012; Meiselman et al. 2018)
	Y	TA			No Effect		Promote sperm release *		(Middleton et al. 2006; Avila et al. 2012; Meiselman et al. 2018)
	Y	5-HT							(Heifetz et al. 2014)
		Proctolin (RYLPT)	Stimulatory		No Effect				(Rodríguez-Valentín et al. 2006; Ritsick et al. 2007; Meiselman et al. 2018)
	Y	Myosupressin							(Heifetz et al. 2014)
***Locusta migratoria* (Orthoptera)**	Y	Glutamate	Stimulatory						(Lange et al. 1984)
	Y	OA	Inhibitory	cAMP			Stimulates contraction	cAMP	(Orchard and Lange 1985; Lange and Orchard 1986; Clark and Lange 2003)
	Y	TA	Inhibitory				Stimulates contraction		(Donini and Lange 2004; da Silva and Lange 2008)
	Y (Spermathecae only)	5-HT	Stimulatory				Stimulates contraction	cAMP	(Clark and A. Lange 2002; Lange 2004)
	Y	Proctolin (RYLPT)	Stimulatory	Ca2+, CAM			Stimulates contraction		(Lange et al. 1986; Lange et al. 1987; Lange

Species (Order)	Confirmed Present in female RT	Neuroactive Molecule	Effect on Oviduct contraction	Secondary Messenger	Effect on Ovary contraction	Secondary Messenger	Effect on SSO	Secondary Messenger	Source
									1988; Lange and Tsang 1993; Nykamp et al. 1994)
	Y	SchistoFLRFamide (myosuppressin)	Inhibitory	reduce Ca2+			Inhibits contraction		(Lange et al. 1991; Peeff et al. 1993; Schoofs et al. 1993; Wang et al. 1994; Wang, Orchard, et al. 1995; Wang, Lange, et al. 1995; Clark and A.B. Lange 2002)
	Y	MIP (B-allatostatin)	Inhibitory				No Effect (Allatostatin)		(Schoofs et al. 1991; Schoofs et al. 1996; Lange and da Silva 2007)
		DA	No Effect						(Lange and Orchard 1983)
	Y (Spermathecae only)	CCAP	Stimulatory				Stimulates contraction		(Donini et al. 2001; da Silva and Lange 2006)
***Rhodnius prolixus* (Hemiptera)**		OA	Inhibitory	cAMP					(Hana and Lange 2017a)
		TA	Inhibitory						(Hana and Lange 2017a)
	Y	Proctolin (RYLPT)	Stimulatory				Stimulates contraction		(Lange 1990; Orchard et al. 2011)
		myosuppressin *	No effect						(Ons et al. 2011; Lee et al. 2012; Sedra et al. 2015)
	Y	MIP (B-allatostatin)	Inhibitory						(Lange et al. 2012; Sedra et al. 2015)
	Y	AST-2 (A-allatostatin)	Inhibitory						(Sedra et al. 2015)
	Y	AKDNFIRFamide (FLP)	Stimulatory		Stimulatory				(Sedra and Lange 2014)

Species (Order)	Confirmed Present in female RT	Neuroactive Molecule	Effect on Oviduct contraction	Secondary Messenger	Effect on Ovary contraction	Secondary Messenger	Effect on SSO	Secondary Messenger	Source
	Y	GNDNFMRFamide (FLP)	Stimulatory		Stimulatory				(Sedra and Lange 2014)
***Tenebrio molitor* (Coleoptera)**		OA	Inhibitory						(Chowanski et al. 2017)
		TA	Inhibitory						(Chowanski et al. 2017)
		DA	Stimulatory						(Chowanski et al. 2017)
		pyrokinin-1	inhibitory						(Marciniak et al. 2012)
		pyrokinin-2	Stimulatory						(Marciniak et al. 2012)
		pyrokinin-3	Stimulatory						(Marciniak et al. 2012)
***Stomoxys calcitrans* (Diptera)**		Glutamate	Stimulatory		Inhibitory				(Cook and Peterson 1989; Cook and Wagner 1992)
		OA	Inhibitory		Stimulatory				(Cook and Peterson 1989; Cook and Wagner 1992)
		Proctolin (RYLPT)	Stimulatory		Stimulatory				(Cook and Peterson 1989; Cook and Wagner 1992)
***Periplaneta americana* (Blattodea)**	Y	OA	Inhibitory	cAMP					(Orchard and Lange 1987; Bamji and Orchard 1995)
	Y	Proctolin (RYLPT)							(Orchard and Lange 1987)
		5-HT	Stimulatory						(Bamji and Orchard 1995)
***Gryllus bimaculatus* (Orthoptera)**		OA	Stimulatory	Ca2+					(Tamashiro and Yoshino 2014)

*denotes effect not linked to muscle activity.