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Current Opinion in Insect Science

The neural basis for insect pheromonal communication

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Insects rely on chemosensory signals to drive a multitude of behavioral decisions. From conspecific and mate recognition to aggression, the proper detection and processing of these chemical signals — termed pheromones — is crucial for insects' fitness. Although the identities and physiological impacts of diverse insect pheromones have been known for many years, how these important molecules are perceived and processed by the nervous system to produce evolutionarily beneficial behaviors is still mostly unknown. Here we present an overview of the current state of research into the peripheral and central nervous system mechanisms that process and drive behavioral responses to diverse pheromonal cues.

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Current Opinion in Insect Science 2015, **12**:86–92

This review comes from a themed issue on **Neuroscience**

Edited by **Yehuda Ben-Shahar**

http://dx.doi.org/10.1016/j.cois.2015.09.010

Introduction

Social communication in insects largely relies on chemosensation. This is likely due to the small body size of insects, which limits their ability to produce and perceive auditory and visual signals, especially over large distances [1]. Chemicals involved in animal communication, known as semiochemicals, can be classified into two categories: pheromones and allelochemicals. Pheromones are chemicals produced and secreted by an organism that elicit a behavioral or physiological response in a member of the same species that receives the signal, while allelochemicals are those that elicit a response in a member of a different species [2]. Recent advances in insect genomics, molecular genetics, and neuroanatomical techniques can now be exploited to understand the mechanisms behind chemical communications in insects. This review will specifically focus on the genetic and neuronal mechanisms that support pheromonal communication in insects.

Insect pheromones and associated behaviors

Throughout their long evolution, insects have co-opted diverse classes of chemicals such as ketones, aldehydes, and fatty acids to serve as pheromones [3]. For example, cuticular hydrocarbons (CHCs) originally evolved as anti-desiccants but now serve a dual role in pheromone signaling [4]. Just as chemicals can vary in their intrinsic properties, such as volatility, so too can pheromones. Consequently, insects have evolved sophisticated pheromone-sensing organs for volatile and non-volatile chemicals. Whereas volatile pheromones, such as ketones, are detected via olfactory receptors housed in the antennae and maxillary palps, low-volatile or non-volatile pheromones, such as long-chain CHCs, are detected via contact chemosensory receptors distributed across the body of the insect [5••,6•].

Like in many other animal lineages, one of the most important functions of pheromones is to drive behaviors associated with mating. Insect mating pheromones are diverse, with different species having evolved the use of different classes of pheromones. For example, Lepidoptera (butterflies and moths), which use pheromones for long-distance sexual advertisement, tend to rely primarily upon volatile compounds [1]. By contrast, fruit flies in the *Drosophila* species group, which use pheromones in complex courtship rituals, exploit both high-volatile and low-volatile CHCs [7]. In insects with dual parental care, pheromones are also used to recognize mating partners [8]. For example, burying beetle females recognize their mate via non-volatile CHC pheromones [9,10]. Pheromones also regulate male–male interactions such as aggression. One such well studied case in *Drosophila melanogaster* involves the pheromone 11-cis-vaccenyl acetate (cVA), a male specific volatile pheromone, which acts pleiotropically to both suppress male–male courtship and elicit male–male aggression [11].

Distinct from mating and sexual behaviors, aggregation pheromones are signals that induce the formation of groups of conspecifics [12]. Aggregation pheromones that act over long-distances are typically volatile and sensed by the olfactory system [13]. By contrast, the cockroach, *Periplaneta americana*, which aggregates during its diurnal resting phase, uses both high-volatile and low-volatile CHCs for the attraction to an aggregation site and the subsequent maintenance of aggregation behavior, respectively [14]. Additionally, pheromone-driven social behaviors that are independent of mating are common in social insects, which include nestmate recognition, and nest

defense, in which volatile alarm pheromones recruit conspecifics to attack intruders [2,15].

Pheromone detection by olfactory systems

The primary insect sensory organs that detect volatile ligands are the antennae and maxillary palps. These organs are covered by an array of anatomically and functionally diverse sensilla that house olfactory receptor neurons (ORNs) tuned to the detection of various chemicals [16]. For example, the silkmoth *Bombyx mori* has four different types of sensilla on its antennae, three of which are tuned to general, non-pheromone chemicals and one of which — the long trichodea — is tuned to the sex pheromones bombykol and bombykal [17]. Similarly, the trichoid sensilla in *D. melanogaster* are specifically tuned to volatile pheromones such as cVA and methyl laurate (ML) [18].

Although the molecular identities of diverse volatile pheromones from many different insect species are known, the receptor proteins that specifically detect them are still mostly undetermined. With the advances of molecular genetics in *Drosophila* and other insects, this gap is now slowly being filled. Based on genetic, neurophysiological, and behavioral studies, two different families of olfactory receptors are likely to detect the majority of insect volatile pheromones. The first identified volatile pheromone receptors were members of the olfactory receptor (*Or* genes) family [5••,19••]. By using neuronal and behavioral approaches, it was shown that cVA can both activate and inhibit innate behavioral programs via the activation of *Or67d*-expressing and *Or65a*-expressing neurons, respectively [20,21]. Furthermore, these neuronal and genetic architectures seem to be conserved across the *Drosophila* species group [5••,18,22,23•]. Like all olfactory receptor neurons, pheromone receptor neurons synapse with central projection neurons in discrete glomeruli within the antennal lobe (AL, Figure 1a). The processing of pheromone signals in the CNS will be further discussed below.

The *Ionotropic receptors* (*Ir*s) comprise the other family of evolutionarily conserved chemoreceptors that detect volatile ligands [24••]. One family member, *IR84a*, has been implicated in promoting male courtship via olfactory pathways [25]. More recently, the *Ir20a* clade of this receptor family has been shown to be enriched in gustatory-like sensilla in the foreleg tarsi and wing margin, which can be activated by non-volatile CHCs. These data suggest that some *Ir* genes also act as receptors that likely respond to contact pheromones, which we discuss in the next section [26•].

Pheromone detection by contact chemosensory systems

Similar to the olfactory system, contact pheromones are detected by chemosensory neurons within gustatory sensilla located on the labellar and tarsal taste organs (Figure 1b). Whereas volatile pheromones are detected by ORNs that express particular odorant receptors, contact pheromones are detected by gustatory receptor neurons (GRNs) that express a variety of contact chemosensory receptors. These include members of the *Gustatory receptor* (*Gr*), *Ir*, and Degenerin/epithelial sodium channel (DEG/ENaC) gene families.

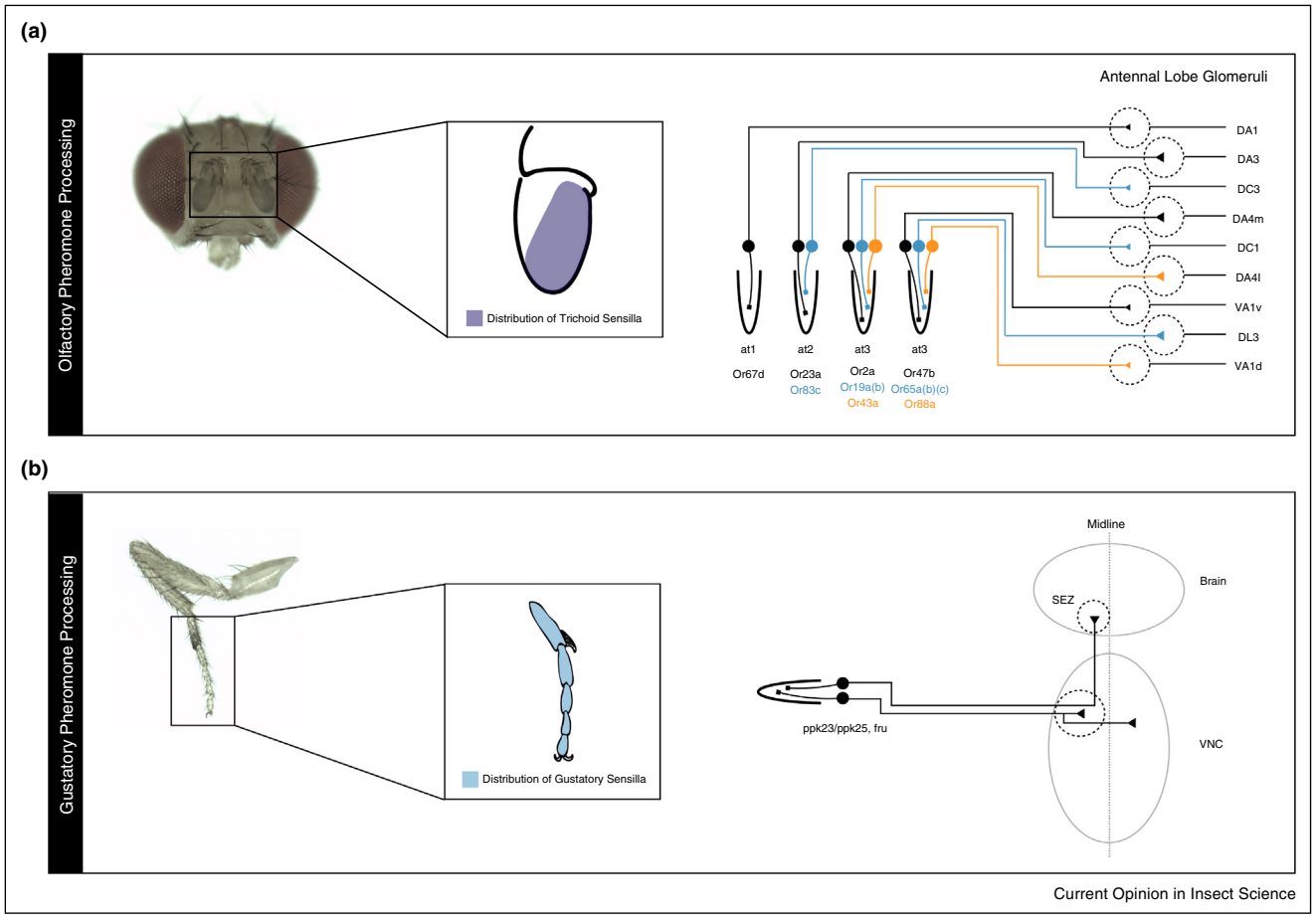
The genes that encode members of the *Gr* family are part of a seven-transmembrane superfamily of insect chemosensory receptors, which also include the *Or* family [6•,27]. Several *Gr* family members — *Gr68a*, *Gr32a*, *Gr33a*, and *Gr39a* — have been implicated in pheromonal communication in *Drosophila* [6•,28–30,31••]. Although the actual pheromone ligands that activate these receptors are not known, *Gr68a* was recently implicated in the perception of CH503, a male-specific *Drosophila* inhibitory contact pheromone [29]. The emergence of state-of-the-art tools for the analysis of neuronal circuits in the fly brain has begun to allow the mapping of various pheromone sensing neurons onto behaviorally relevant circuits, shedding light on how they might trigger male-specific and female-specific mating behaviors. For example, at least one study has recently investigated a circuit-level mechanism for how inhibitory contact pheromones may suppress male courtship via the activation of *Gr32a*-expressing sensory neurons [32].

In contrast to vertebrates, which mostly use metabotropic dependent signaling pathways for chemosensation, including pheromonal communication [33], the insect chemoreceptor repertoire is mostly ionotropic [34]. Consequently, it has been speculated that other large families of ion channels might serve as chemoreceptors in insects. One of the largest ion channel families in the *Drosophila* genome is the DEG/ENaC family, named *pickpocket* (*ppk*) genes [35]. Not surprisingly, recent studies have suggested that the ion channels encoded by *ppk23*, *ppk25*, and *ppk29* play a role in chemosensory functions associated with mating behaviors in *Drosophila* [36••,37–40]. However, whether *ppk* channels exert their function by directly acting as receptors for pheromone ligands — or indirectly as co-receptors — has not been demonstrated to date.

Pheromonal circuits responsible for processing pheromones

Much of what we know about the action of insect pheromones is in the context of mating behaviors. In spite of the immense pheromonal diversity exhibited by insects, almost all we know about the central neuronal processing of pheromonal signals comes from a single insect species, the fruit fly *D. melanogaster*. In this species, the sexual identity of the neuronal circuit associated with mating behaviors is determined by the well-characterized sex-determination factor *fruitless* (*fru*). Sexually dimorphic

Figure 1



Anatomy of select olfactory and gustatory organs in the fly. **(a)** The antennae contain trichoid sensilla that are important for the detection of volatile pheromones. Trichoid sensilla are distributed across the antennae, but are more concentrated along the lateral edge of the antennae. There are four different classes of trichoid sensilla (at1–at4) that house ORNs expressing different olfactory receptors. The particular ORs that are expressed within each sensilla are listed below its respective sensillum. ORNs are shown above each sensillum projecting into their corresponding glomeruli within the AL. **(b)** Processing of contact pheromones in the forelegs of male flies occurs within gustatory sensilla that co-express *ppk23* and *fruitless*. These GRNs send axonal projections into both the prothoracic neuromere of the VNC (dotted circle) and the sub-esophageal zone of the brain. Within the VNC, some *ppk23/fru* neurons send projections across the neural midline.

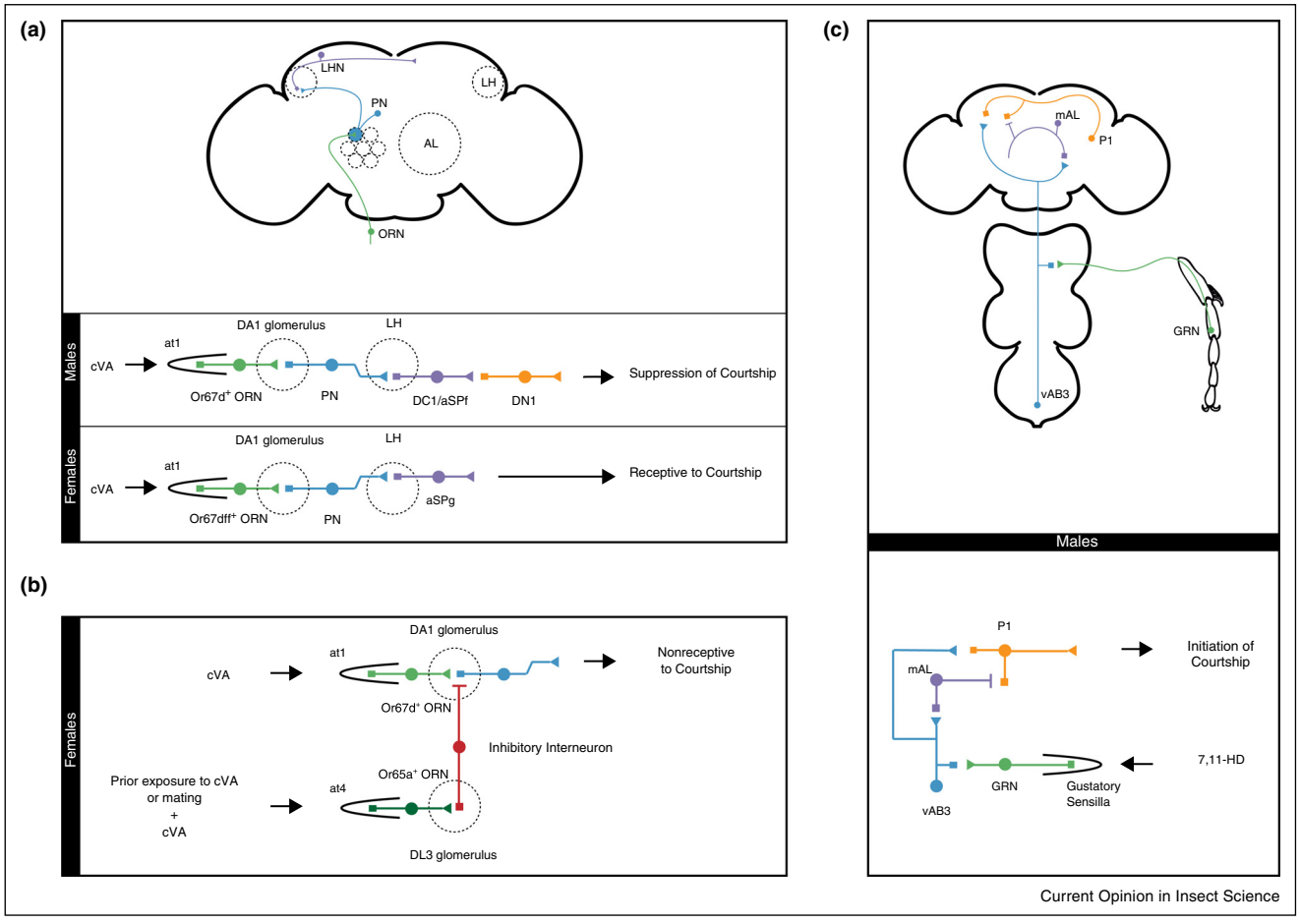
splicing of the *fru* mRNA in ~1500 neurons in the central and peripheral nervous systems is sufficient to drive male-specific versus female-specific neuronal development [41] and mating behaviors [42,43]. In the peripheral nervous system, both ORNs and GRNs that have been shown to respond to pheromones also express *fru*. For instance, cVA-responsive ORNs in the antennae and contact-pheromone-responsive GRNs in the foreleg tarsi both express *fru* [36••].

The action of the *Drosophila* pheromone cVA is well understood in molecular and cellular terms. Consequently, much of the effort to decipher neuronal circuits that mediate the impact of pheromones on behavior has focused on this volatile pheromone. The male-specific cVA is produced by the ejaculatory bulb and transferred to females during courtship and copulation. Accordingly, cVA has been implicated in diverse mating-related functions such as suppression of male–male courtship, induction of male–male aggression, increases in virgin female sexual receptivity, and suppression of post-mating female attractiveness [11,20,44]. cVA is detected by *fru*-expressing ORNs that are housed in T1 trichoid sensilla of the third antennal segment and express the receptor *Or67d* [45•]. These neurons project their axons to the sexually dimorphic DA1 glomerulus of the antennal lobe where they synapse with secondary projection neurons (PNs) that then project to the lateral horn (LH) of the protocerebrum [20,46,47]. By using both high-resolution morphological imaging and *in vivo* electrophysiological recordings, these studies were able to identify the tertiary neurons that are activated by cVA [46,48]. In particular, careful analyses of the cVA circuit revealed that DA1 PNs communicate with a different subclass of LH neurons in

each sex, which indicates that pheromone signal processing at this third-order synapse supports the alternative, cVA-dependent behavioral outputs between males and females (Figure 2a). These reports were the first to indicate how a single pheromone can lead to sexually dimorphic behavioral responses via alternative signal processing within the central nervous system.

Although cVA is initially detected by *Or67d*-expressing ORNs and processed so that it has an aphrodisiac effect in females, the behavioral valence of cVA can change over time depending on mating status. Particularly, whereas cVA is attractive to virgin females, it is repulsive to females post-mating [5••]. The neural basis for the alternative effects of this pheromone on female behavior was recently shown to result from the chronic, cVA-mediated activation of an additional class of ORNs that express *Or65a* [8,23]. These studies suggest a mechanism whereby *Or65a*-expressing neurons inhibit *Or67d*-expressing output in the AL via inhibitory interneurons that connect

Figure 2



Circuits important for processing pheromones in the fly. **(a)** Sexually dimorphic circuitry connecting ORNs expressing *Or67d* to higher order processing centers in the brain lead to alternative behavioral responses to cVA in males and females. An overview of the neurons important for processing cVA is shown above detailed circuit maps for males and females. cVA is detected by *Or67d*-expressing ORNs present in at1 sensilla. These ORNs send projections into the DA1 glomerulus of the AL where they synapse with projection neurons (PNs) that send axons to the lateral horn (LH). Male PNs synapse in a more ventral region of the LH than female PNs and make functional connections with DC1/aSPf neurons. Female PNs synapse more dorsally within the LH and are functionally connected to aSPg neurons. Finally, a fourth-order neuron (DN1) has been identified in males which likely connects DC1/aSPf with motor neurons in the VNC. The outputs from this sexually-dimorphic circuit are believed to mediate the suppressive or receptive behavioral responses to cVA in males and females, respectively. Circuit model was adapted from [46,48]. **(b)** Chronic cVA exposure or mating also leads to an inhibitory effect of cVA on glomeruli in the female AL. In particular, pre-exposure to cVA — detected by *Or65a*-expressing ORNs present in at4 sensilla — leads to activation of the DL3 glomerulus. Inhibitory interneurons are thought to connect the DL3 and DA1 glomeruli in the AL. Thus activation of the DL3 glomerulus leads to inhibition of the DA1 glomerulus in mated females, which leads to a decrease in receptivity to mating. Circuit model was adapted from [23•]. **(c)** The circuit that is important for the detection of 7,11-HD and the initiation of courtship is shown. 7,11-HD is initially detected by *ppk25*-expressing gustatory receptor neurons, which then synapse with *fru*-expressing vAB3 neurons present in the VNC. These vAB3 neurons project to the brain and directly activate P1 neurons that are required for courtship initiation. vAB3 neurons also are functionally connected to mAL neurons which have an inhibitory effect on P1 neurons and are thought to function as a gain control mechanism to maintain steady levels of P1 excitation. Circuit adapted from [31••].

their respective glomeruli (see Figure 2b for details). Collectively, upon first-exposure to cVA, virgin females are attracted to this pheromone via activation of *Or67d*-expressing ORNs. However, once cVA has been transferred to the cuticle of the female following mating, it chronically activates *Or65a*-expressing ORNs. This suppresses output from *Or67d*-expressing ORNs, and thus reduces attraction toward this chemical. These results have helped to lend further insight into how the alternative processing of a single pheromone can produce distinct behavioral outputs.

Much of what we know about the neuronal processing of pheromonal signals comes from studies of a single pheromone, cVA. However, many different pheromones can interact to drive a particular behavioral response [49]. Consequently, understanding how pheromones drive behavior requires determining how signals from multiple different pheromones are integrated to induce relevant, adaptive behaviors. As a first step in this direction, a recent study has identified a neural circuit that integrates the olfactory processing of cVA with the processing of the non-volatile contact pheromones 7,11-heptacosadiene (7,11-HD) and 7-tricosene (7-T) to initiate courtship in males [31••]. Typically, a wild type male will initiate courtship by approaching and tapping a female using his forelegs to sample the CHCs present on the female's cuticle. Excitatory pheromones on the female — including 7,11-HD — induce activation of a population of *fru*-expressing neurons in the male's brain termed P1 neurons [50]. The activation of this population of cells is not only required for the initiation of courtship, but it is sufficient to induce male courtship even in the absence of a female. An important recent study elucidated the neural circuitry that links sensory reception of 7,11-HD by foreleg GRNs with the activation of P1 neurons in the central nervous system (see Figure 2c for an overview of this circuit). Furthermore, this study demonstrated how cVA detection through *Or67d*-expressing ORNs is linked to P1 neurons and acts to suppress the activity of these cells even in the presence of 7,11-HD, which could explain why cVA has such a strong anti-aphrodisiac effect on males. The interplay of olfactory and gustatory pheromones is likely a common mechanism for regulating innate behavioral decisions, and the cVA-7,11-HD circuitry should help to define a promising model for interrogating these multi-sensory interactions.

Conclusions

In spite of many of years of research into the role of pheromones in the regulation of insect behavior, our understanding of the mechanisms and evolutionary processes that support these complex signals are still in their infancy. Although studies in the fruit fly *D. melanogaster* are paving the way for understanding the sensory, neuroethological, and genetic principles of pheromonal communication, the current lack of comparable genetic tools for other insect species hinders progress in the field. However, recent progress in genome editing techniques promises that in the near future, similar studies could be accomplished in any insect species. The ability to identify receptors and cells responsible for pheromonal communication in diverse insect species will enable the field to take advantage of the wealth of existing behavioral and physiological data from these species to develop a comparative research framework. Such a framework will enable us to better understand insect behavior in evolutionary and neuroethological terms. Furthermore, since many insect species are considered pests or disease vectors, better mechanistic understanding of their pheromonal signaling systems will enable the development of more sustainable and specific methods to control their behavior.

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