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Presynaptic modulation of early olfactory processing in *Drosophila*

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Abstract

Most animals are endowed with an olfactory system that is essential for finding foods, avoiding predators, and locating mating partners. The olfactory system must encode the identity and intensity of behaviorally relevant stimuli in a dynamic environmental landscape. How is olfactory information represented? How is a large dynamic range of odor concentrations represented in the olfactory system? How is this representation modulated to meet the demands of different internal physiological states? Recent studies have found that sensory terminals are important targets for neuromodulation. The emerging evidence suggests that presynaptic inhibition scales with sensory input and thus, provides a mechanism to increase dynamic range of odor representation. In addition, presynaptic facilitation could be a mechanism to alter behavioral responses in hungry animals. This review will focus on the GABA_B receptor-mediated presynaptic inhibition, and neuropeptide-mediated presynaptic modulation in *Drosophila*.

2. Introduction

Mammalian Olfactory System

Several key findings made in the last two decades have greatly enhanced our understanding of the olfactory sense (Bargmann, 2006; Julius and Katz, 2004), particularly the discovery of a large family of some 1,000 different olfactory receptor genes in the mouse genome (Buck and Axel, 1991). Each olfactory receptor neuron (ORN) expresses just one receptor gene (Chess et al., 1994; Malnic et al., 1999) and neurons expressing the same receptor gene converge with high accuracy onto a single glomerulus in the olfactory bulb (Mombaerts et al., 1996; Ressler et al., 1994; Vassar et al., 1994), establishing the notion that the olfactory system employs spatial segregation of sensory input to encode the quality of odors (Buck and Axel, 1991). A single odorant receptor can recognize multiple odorants, and a single odorant typically excites multiple receptors. Hence, by virtue of the combinatorial receptor code (Malnic et al., 1999), the olfactory system has the potential to recognize many more odors than its number of receptor types. Keeping track of synaptic connectivity and modulation poses some of the greatest challenges in studying the complex olfactory system. It is therefore beneficial to have an anatomically simple system that is amenable to both cellular manipulations and functional monitoring of neural activity.

A Simple Olfactory System in *Drosophila*

The identification of a complete family of odorant receptor genes makes the fly an attractive system to study the successive propagation of sensory information. ORNs detect odors in the

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periphery and send axons to the antennal lobe. An adult fly expresses about 60 odorant receptor genes and each ORN typically expresses just one or a few receptor genes (Benton et al., 2009; Clyne et al., 1999; Couto et al., 2005; Fishilevich and Vosshall, 2005; Gao and Chess, 1999; Scott et al., 2001; Vosshall et al., 1999; Vosshall et al., 2000). *Orco*, an olfactory coreceptor gene, is also expressed in many ORNs. *Orco* is required for dendritic localization of the receptor protein (Benton et al., 2006; Larsson et al., 2004). Importantly, the olfactory responsivity of an ORN is determined by expression of the receptor gene and not *Orco* (Benton et al., 2006; Elmore et al., 2003; Hallem et al., 2004; Wang et al., 2003). This numerically simpler olfactory system coupled with genetic markers to label most of the input channels provides an opportunity to study synaptic function and information processing in great detail.

In the antennal lobe, axons of ORNs expressing the same receptor gene project with precision to spatially invariant glomeruli (Benton et al., 2009; Couto et al., 2005; Fishilevich and Vosshall, 2005; Vosshall et al., 2000). A near complete map of ORNs has been established with nearly 60 *Or-Gal4* transgenic lines, providing essential reagents to mark the peripheral olfactory system. Projection neurons (PNs) send dendrites to individual glomeruli in the antennal lobe. There are only a few PNs for each glomerulus (Datta et al., 2008; Vosshall and Stocker, 2007; Wong et al., 2002). PN axons innervate the lateral horn of the protocerebrum and make synaptic connections with Kenyon cells in the mushroom body. The fly antennal lobe is a sphere of 50µm in diameter, and all 50 glomeruli are easily accessible to optical imaging.

The last few years have seen the discovery of stereotypic organization at every level of the *Drosophila* olfactory system, including the antenna (de Bruyne et al., 2001; Hallem and Carlson, 2004), the antennal lobe (Couto et al., 2005; Fiala et al., 2002; Fishilevich and Vosshall, 2005; Laissue et al., 1999; Marin et al., 2002; Ng et al., 2002; Suh et al., 2004; Wang et al., 2003; Wong et al., 2002), the mushroom body (Lin et al., 2007; Tanaka et al., 2004; Wang et al., 2004), and the protocerebrum (Jefferis et al., 2007; Marin et al., 2002; Tanaka et al., 2004; Wong et al., 2002). Knockout of an odorant receptor gene or ectopic expression of a different receptor gene does not alter axonal innervations of the ORNs (Dobritsa et al., 2003; Elmore et al., 2003; Hallem et al., 2004; Root et al., 2007; Wang et al., 2003). Thus, one can use receptor gene mutations to silence ORNs without the complication of developmental disruption. This stereotypic organization simplifies experimental designs to investigate information processing in the antennal lobe.

Many tools are available in *Drosophila* to record neural activity as well as label and manipulate selected neurons within a circuit. Since the invention of the Gal4/UAS system for targeted gene expression (Brand and Perrimon, 1993), thousands of Gal4 lines for different parts of the fly brain have been established including several lines that label different PNs (Stocker et al., 1997; Tanaka et al., 2004). Efforts to identify more fly lines for specific neuron populations in the antennal lobe will facilitate our studies of information processing. Mosaic analyses using MARCM (Lee and Luo, 1999) or Flp-out (Zecca et al., 1996) techniques provide tools to dissect the olfactory circuit with cellular resolution (Jefferis et al., 2007; Lin et al., 2007; Marin et al., 2002; Wong et al., 2002). Also, former difficulties with physiologic recordings have been mitigated by the development of several neural techniques, including single-neuron electrophysiology (de Bruyne et al., 2001; Wilson et al., 2004), and optical imaging with genetically encoded activity indicators (Fiala et al., 2002; Ng et al., 2002; Wang et al., 2003; Wang et al., 2004; Yu et al., 2004). Genetic dissection of this simple olfactory system should provide a productive system to study the synaptic function in defined neural circuits and the mechanisms of information processing in detail.

It is easier to set up favorable experimental conditions to investigate olfactory information processing in *Drosophila* than in mouse. Important differences, however, exist between these long-diverged species (Mombaerts, 1999). For instance, the two odorant receptor gene families of mice and flies have practically no sequence homology. Also unlike flies, receptor genes in the mouse are required for ORN axons to innervate their correct targets during development (Mombaerts et al., 1996). These fundamental differences suggest that the olfactory systems of insects and mammals arose through convergent evolution from independent origins (Strausfeld and Hildebrand, 1999). Nevertheless, whether the substantial similarities reflect homology or convergence, it is likely that what one learns from *Drosophila* about how olfactory information is processed in the antennal lobe will shed new light on the common principles of olfactory computation. Comparative studies should provide general principles of synaptic modulation in basic neural circuits. Recent studies suggest that mechanisms of presynaptic modulations are shared by the olfactory systems of insects and mammals. Furthermore, olfactory information at the periphery reaches the olfactory cortex within two synapses, making olfactory system the shallowest one among all sensory systems and accentuating the importance of neuromodulation at the first synapse.

3. Presynaptic modulation is mediated by diverse G-protein-coupled receptors

Examples of presynaptic modulation

Modulation of early sensory processing can have profound effects on stimulus detection and animal behaviors. For instance, serotonin mediates presynaptic facilitation of mechanosensory neurons in *Aplysia* to sensitize the siphon and gill withdrawal reflex (Brunelli et al., 1976). Pairing siphon stimulation with an electrical shock to the tail results in sensitized gill withdrawal reflex. Electrical shocks cause serotonin release from the interneurons that synapse at the axon terminals of sensory neurons. Activation of the serotonin receptors at the sensory terminals enhances neurotransmitter release to increase synaptic potential of the motor neurons that control gill withdrawal behavior. In another example, feeding leeches release serotonin into the sensory circuit, which suppresses neurotransmitter release from the mechanosensory neurons. The serotonin mediated presynaptic inhibition of mechanosensory transmission establishes a behavioral hierarchy in which feeding suppresses tactile sensation (Gaudry and Kristan, 2009).

GABAergic interneurons in the olfactory system

The olfactory system is rich with GABAergic interneurons. In the lateral inhibition hypothesis each glomerulus inhibits its neighbors via interneurons that connect multiple glomeruli. This notion has received support from studies of different olfactory systems (Isaacson and Strowbridge, 1998; Mori et al., 1999; Sachse and Galizia, 2002). In insects, the role of local interneurons in the antennal lobe has been investigated in detail. An electron microscopy study of the cockroach antennal lobe showed that ORNs are connected to output PNs in two different ways: a direct monosynaptic connection and a polysynaptic connection via the GABAergic interneurons (Distler and Boeckh, 1997). GABA, released by interneurons, hyperpolarizes PNs in two different ways. The fast hyperpolarization is mediated by GABA_A receptor, which is sensitive to the antagonists picrotoxin and bicuculine (Christensen et al., 1993; Christensen et al., 1998; Wilson et al., 2004; Wilson and Laurent, 2005). The slow hyperpolarization is mediated by GABA_BR, which is sensitive to the GABA_BR antagonist CGP54626 (Wilson and Laurent, 2005).

GABA_B receptor-mediated presynaptic inhibition

GABA_B Receptors—GABA_B receptors (GABA_BRs) are major sites of slow synaptic inhibition in the central nervous system. Activation of presynaptic GABA_BRs inhibits voltage-dependent calcium channels via the $\beta\gamma$ G-protein complex, and consequently suppresses neurotransmitter release (Bettler et al., 2004). This presynaptic inhibition mechanism has been demonstrated in the mammalian olfactory system (McGann et al., 2005) and hippocampus (Isaacson et al., 1993; Thompson et al., 1993). The low sensitivity of presynaptic inhibition to stimulus intensity is often explained by the GABA spillover model (Isaacson et al., 1993; Scanziani, 2000), which proposes that the simultaneous release of GABA by several interneurons is necessary to activate GABA_BRs. This model is consistent with the observation that GABA_BRs are often distant from the GABA releasing sites (Mody et al., 1994). Activation of postsynaptic GABA_BR in dendritic spines controls glutamatergic transmission via GIRK channels, which are the main postsynaptic effectors of GABA_BR. The GIRK-mediated slow hyperpolarizing currents in dendritic spines are important for the induction of synaptic plasticity (Otmakhova and Lisman, 2004) and the slow currents can also exhibit long-term change (Huang et al., 2005). It has become clear in recent years that changes in the function of GABA_BRs play a key role in drug addiction, epilepsy, nociception and schizophrenia (Bettler et al., 2004). GABA_B metabotropic receptors found in mouse ORNs (Kratskin et al., 2006) mediate the delayed feedback inhibition of ORN output (Wachowiak et al., 2005). It has been hypothesized that feedback inhibition provides a mechanism for modulating input sensitivity, attenuating post-synaptic responses during repeated sniffing, and adaptive filtering of the odor landscape (Aroniadou-Anderjaska et al., 2000; Duchamp-Viret et al., 2000; McGann et al., 2005; Verhagen et al., 2007).

GABA_B receptor-mediated gain control: Two recent studies in *Drosophila* show that GABA_BRs at axon terminals of ORNs mediate presynaptic inhibition to control olfactory sensitivity. The fruit fly has two olfactory organs: the antenna, which contains 90% of all ORNs, and the maxillary palp, which houses the rest. Olsen and Wilson used whole patch clamp recordings to measure olfactory responses in PNs associated with VM7, a glomerulus that receives direct ORN input from the palp (Olsen and Wilson, 2008). Severing the antennal nerve therefore eliminates most of the lateral input into the VM7 glomerulus. They found that reduced lateral input increases the spike activity of VM7 PNs, and the effect of antennal removal can be mimicked by applying two antagonists, one for the GABA_AR and the other for the GABA_BR (Figure 1A and B). Additional evidence corroborating this finding comes from experiments where Root and colleagues measured neural activity directly from sensory terminals (Root et al., 2008). Targeted genetic expression of the calcium sensor GCaMP and the pH sensor synaptopHluorin permits the monitoring of ORN terminal calcium and vesicle release, respectively. GABA and the GABA_BR agonist SKF97541 suppress calcium activity and neurotransmitter release. The effect of GABA is prevented by bath application of the GABA_BR antagonist CGP54626 or targeted RNA interference mediated knockdown of *GABA_BR2* in ORNs. Furthermore, activation of LNs suppresses terminal calcium and this suppression is prevented by CGP54626. These observations from optical imaging are in concordance with those from the electrical recording study, establishing the model that LNs release GABA, which activates the GABA_BRs in presynaptic terminals to suppress calcium influx and subsequent neurotransmitter release from sensory neurons. Because the response of LNs scales with ORNs' response, this presynaptic inhibition also scales with sensory response (Figure 1C). Thus the gain control mechanism mediated by presynaptic GABA_B receptors leads to a slope change in the input-output plot of olfactory responses (PN firing rate, presynaptic calcium, synaptic release) to varying odor concentration.

Do GABA_ARs mediate presynaptic inhibition in the *Drosophila* antennal lobe? Two recent studies offer seemingly contradictory answers (Olsen and Wilson, 2008; Root et al., 2008). Whole-cell recordings of PNs suggest that GABA_ARs and GABA_BRs mediate the early and late phase inhibition, respectively (Olsen and Wilson, 2008). Results from optical monitoring of presynaptic calcium indicate that picrotoxin does not block the effect of GABA on presynaptic calcium, suggesting that GABA_A receptors do not play any role in this presynaptic inhibition (Root et al., 2008). One potential explanation may account for these seemingly contradictory observations. It is possible that the early phase effect of picrotoxin on the spike activity of PNs is through a polysynaptic pathway to PNs. For example, some GABAergic LNs could synapse with a second population of LNs that make excitatory synapses with the PNs. In this polysynaptic system, olfactory input could suppress PNs through a picrotoxin-sensitive synapse.

Root and colleagues also discovered heterogeneity in the levels of presynaptic inhibition among different glomeruli (Root et al., 2008). Varying GABA_BR2 expression level in ORNs with molecular manipulations is sufficient to produce predictable alterations in presynaptic inhibition. Therefore, presynaptic GABA_BR expression level is a determinant of glomerulus-specific olfactory gain in the antennal lobe, arguing that PN response is not a faithful transformation of receptor input (Figure 1C). GABA_BR-mediated presynaptic inhibition may provide a mechanism to expand the dynamic range of olfactory responses. However, there is a tradeoff between dynamic range and sensitivity. Sex pheromones are important olfactory cues for flies to find mating partners (Greenspan and Ferveur, 2000; Hall, 1994). Having a large dynamic range in these glomeruli is important for male flies to navigate towards a female fly. For aversive odors such as CO₂, detection sensitivity is a more important feature (Suh et al., 2007; Suh et al., 2004). Indeed, the CO₂ sensing glomerulus is devoid of the GABA_BR and the pheromone sensing glomeruli DA1 and VA11m exhibit high levels of GABA_BRs (Figure 1C). The importance of olfactory dynamic range is further supported by the finding that genetic knockdown of GABA_BRs in the VA11m glomerulus reduces the ability of male flies to locate female flies in a behavioral assay (Figure 1D).

Unlike flies, GABA_BR-mediated presynaptic inhibition in mice does not scale with ORN input (Pirez and Wachowiak, 2008). Receptor input strength is tonically inhibited by second-order neurons in the glomerular layer. It has been speculated that the strength of presynaptic inhibition in the mouse is controlled by behavioral state. Thus, the relevance of GABA_BR-mediated presynaptic inhibition to mouse olfactory behaviors warrants further studies.

Serotonin modulation of presynaptic inhibition: Serotonin plays an important modulatory role in shaping sensory representations during wakefulness (Hurley et al., 2004). In mammals, serotonergic neurons in the brainstem raphe nuclei are quiet during sleep and active during wakefulness (Jacobs and Fornal, 1991). Raphe neurons send many axons to the olfactory bulb (McLean and Shipley, 1987). Similarly, serotonin levels in the antennal lobe in insects vary throughout the day, reaching their peak when insects are most active and hitting their trough when insects are least active (Kloppenborg et al., 1999). A survey of 40 insect species including *Drosophila* shows that centrifugal serotonergic neurons exhibit elaborate innervation patterns in the antennal lobe (Dacks et al., 2006). Therefore, arousal states may modulate olfactory processing in the first synapse via the levels of serotonin.

Two recent studies in mouse and *Drosophila* have investigated how serotonin modulates odor representations in the early olfactory circuit (Dacks et al., 2009; Petzold et al., 2009). Petzold and colleagues used two-photon microscopy to monitor olfactory inputs to olfactory bulb glomeruli in mice (Petzold et al., 2009). They measured olfactory inputs by genetically

expressing synaptopHluorin—a probe of vesicle release—in ORNs (Figure 2A). They found that activation of the 5-HT_{2C} receptor in juxtaglomerular neurons suppresses ORN transmission release in response to odor stimulation (Figure 2B). The same suppression is also achieved by electrical stimulation of the raphe nuclei. These results suggest that mice in arousal states have attenuated olfactory inputs into the olfactory bulb. A similar reshaping of olfactory representations by serotonin in the early olfactory system has been found in *Drosophila* by Dacks and colleagues (Dacks et al., 2009). They employed two-photon microscopy to monitor synaptic transmission with spH and intracellular calcium with GCaMP. Serotonin enhances the response of local interneurons (Figure 2C), resulting in more suppression of neurotransmitter release from presynaptic ORNs. Dacks and colleagues further demonstrated that serotonin does not simply dampen olfactory sensitivity, because some postsynaptic PNs exhibit increased sensitivity to odor stimulation (Figure 2D). This suggests that PNs may express 5-HT receptors as well. When serotonin is released into the antennal lobe, it reduces ORN input but increases PN excitability with an end result of more neural activity in antennal lobe output PNs.

Tachykinin and presynaptic inhibition

Recent studies have discovered a third mechanism for presynaptic inhibition in the *Drosophila* antennal lobe (Ignell et al., 2009). *Drosophila* tachykinin (DTK) binds to its receptors (DTKRs) at sensory terminals to suppress calcium influx and neurotransmitter release. The neuromodulatory role of tachykinin has attracted some attention because of its fluctuation with nutritional status. Upon starvation, the midgut of locust and cockroach releases tachykinin into circulation (Winther and Nassel, 2001).

Cellular communication using the tachykinin family of neuropeptides and their receptors is an evolutionarily ancient mechanism. DTKR bears high homology with the mammalian tachykinin receptor family that includes NK1, NK2 and NK3 (Li et al., 1991). The tachykinin neuropeptides substance P, neurokinin A and neurokinin B preferentially activates NK1, NK2 and NK3, respectively. Interestingly, mammalian substance P but not neurokinin A or neurokinin B is capable of activating DTKR when the receptor is expressed in *Xenopus* oocytes (Li et al., 1991). In mammals, substance P is the most abundant tachykinin in the CNS (Berton and Nestler, 2006). The role of substance P and NK1 in peripheral pain sensitivity has been well documented. Inflamed tissues produce increased amounts of NGF, which in turn upregulates the expression of substance P in nociceptors. Activation of the NK1 receptor by substance P in second-order neurons in the spinal cord is involved in the hyperalgesia that accompanies inflammation (Hunt and Mantyh, 2001). Furthermore, substance P and NK1 have also been implicated in anxiety and depression in humans. In response to emotional stressors, rats exhibit long lasting release of substance P in the medial nucleus of the amygdala; microinjection of an NK1 antagonist reduces anxiety-related behaviors (Ebner et al., 2004). Despite the tremendous progress in the last few decades of research into the role of substance P and NK1 in chronic pain, depression and anxiety, therapeutic use of NK1 antagonists has met with many difficulties (Berton and Nestler, 2006). Studying the functional role of tachykinins and their receptors in defined neural circuits in a simpler nervous system such as *Drosophila* may provide new insights into their evolutionarily conserved biological functions.

Two tachykinin receptors, NKD and DTKR, have been identified in *Drosophila* based on their sequence similarity to the mammalian tachykinin receptors (Li et al., 1991; Monnier et al., 1992). All vertebrate tachykinins share the pentapeptide sequence FXGLM in the C-terminal, while invertebrate tachykinins contain the pentapeptide FX₁GX₂R (Nassel and Winther, 2010). Nevertheless, mammalian substance P is capable of activating DTKR, although *in vivo* measurements indicate that *Drosophila* tachykinin activates DTKR at lower concentrations than substance P (Birse et al., 2006). Winther and colleagues hypothesized

that tachykinin signaling plays an important role in olfactory perception in *Drosophila*, based on the observation that the antennal lobe exhibits the highest expression levels of tachykinin in the adult brain (Winther et al., 2006). Using RNAi to knockdown the expression of the gene encoding the tachykinin precursor, they demonstrated that tachykinin signaling contributes to innate odor preference in a T-maze test. How does tachykinin signaling modulate the olfactory circuit to influence odor perception? Ignell and colleagues used a multidisciplinary approach that integrates two-photon calcium imaging with molecular perturbations and a behavioral assay to investigate the underlying neural circuitry (Ignell et al., 2009). Immunostaining with an antiserum against a cockroach tachykinin reveals that some GABAergic LNs in *Drosophila* antennal lobe express tachykinin, suggesting that some LNs release GABA and tachykinin as a cotransmitter. RT-PCR and immunostaining experiments show that *Drosophila* ORNs express DTKR at axon terminals. Tachykinin suppresses calcium activity of ORN terminals in response to the stimulation of olfactory sensory neurons. Furthermore, using synaptopHluorin to measure vesicle release from ORNs, they found that tachykinin suppresses vesicle release. This suppression by tachykinin is abolished in flies that express DTKR-RNAi in ORNs. These observations suggest that tachykinin mediates a presynaptic inhibitory feedback from LNs to suppress ORN neurotransmitter release. A careful comparison of the glomerular activity in response to two different odorants reveals that the DM1 glomerulus exhibits greater responses in DTKR knockdown flies than that of the control flies. This alteration in DM1 response explains why DTKR knockdown flies are more attracted to the two odorants, because DM1 is shown in a recent report as a glomerulus that mediates olfactory attraction behavior (Semmelhack and Wang, 2009).

Ignell and colleagues also show that GABA_BRs and DTKRs in sensory neurons independently mediate presynaptic inhibition to suppress ORN neurotransmitter release (Ignell et al., 2009). The source of GABA and tachykinin can come from the same LNs in the antennal lobe. This appears to be two redundant mechanisms for presynaptic inhibition. However, it remains to be determined whether the levels of GABA_BRs or DTKRs will change in different behavioral context and whether the levels of GABA or tachykinin will vary as well. Also it will be interesting to investigate whether and how substance P modulates olfactory sensitivity in mammalian olfactory bulb, since substance P immunoreactivity is present in the short axon cells (Davis et al., 1982).

sNPF Receptor-mediated Presynaptic Facilitation and Food Search Behavior

Starvation Modulation—The modulation of behavior by internal physiological states is essential for animal survival (Barsh and Schwartz, 2002). Hungry animals exhibit different food-searching (appetitive) and food-intake (consummatory) behaviors in order to maintain energy homeostasis (Dethier, 1976). Olfaction is an ancient sense that makes important contributions to the perception of food quality and dietary selection (Shepherd, 2006). Learning how olfactory neural circuits impact dietary choices is relevant towards better understanding factors that contribute to obesity as well as anorexia in the infirm and elderly. Like many other animals, fruit flies exhibit different food searching and food-intake behaviors upon starvation (Douglas et al., 2005). The sense of smell plays an important role in appetitive behavior in *Drosophila* (Semmelhack and Wang, 2009). Not all glomeruli responding to cider vinegar, a food odor for *Drosophila*, are required for the fly's innate attraction towards low concentrations of cider vinegar; nor are all glomeruli responsive to cider vinegar required for the fly's innate aversion to high concentrations of cider vinegar. The DM1 glomerulus mediates attraction behavior and the DM5 glomerulus mediates avoidance behavior. Similar findings from studies of the mouse olfactory system lend support to the notion that select glomeruli are genetically hardwired for innate odor preferences (Kobayakawa et al., 2007). In a recent study, we show that starved flies exhibit

increased responses in the DM1 glomerulus (Figure 3A–C). This suggests the odor map is fine-tuned by the internal state of the animal.

How does starvation retune the odor map? It has been well established that neuromodulators exert their influence over CNS targets by altering neuronal excitability and/or network properties (Destexhe and Marder, 2004; Katz and Frost, 1996; Kupfermann, 1979). Important progress has been made in identifying key hormones and neuromodulators that serve to communicate an animal's nutritional status to the CNS and thus, change the animal's behavior (Barsh and Schwartz, 2002; Berthoud, 2002; Magni et al., 2009).

Neuropeptide NPY/NPF—Microinjection of neuropeptide Y (NPY) into the hypothalamus increases food-intake in mammals (Stanley et al., 1992). Neuropeptide F (NPF) and short neuropeptide F (sNPF) are two insect homologs of the mammalian NPY (Brown et al., 1999; Hewes and Taghert, 2001; Lee et al., 2004; Nassel and Winther, 2010). In *Drosophila*, over-expression of the *NPF* or *sNPF* genes similarly increases the animal's size (Lee et al., 2004; Wu et al., 2003). Interestingly, the olfactory epithelia of mouse (Hansel et al., 2001), zebrafish (Mathieu et al., 2002) and salamander (Mousley et al., 2006) all exhibit NPY immunoreactivity, and *Drosophila* ORNs express sNPF (Carlsson et al., 2010; Nassel et al., 2008). A recent study shows that targeted expression of RNAi in *Drosophila* ORNs to knockdown sNPF or its receptor gene (sNPFR1) blocks the starvation-enhanced DM1 response to food odors and reduces efficiency of food search behavior (Root et al., 2011).

Insulin—Insulin and the insulin receptor (InR) function to promote glucose uptake in both vertebrates and invertebrates (Kim and Rulifson, 2004; Rulifson et al., 2002). Behaviorally, insulin signaling promotes satiety in rodents (Bruning et al., 2000; Woods et al., 1998). In *Drosophila*, insulin is produced in the pars intercerebralis (Rulifson et al., 2002). In adult vertebrates, insulin is produced by the pancreas and crosses the blood brain barrier to reach the CNS. Results from a recent study show that constitutive activation of InR in ORNs, prevents enhancement of food seeking behaviors in fasted animals (Root et al., 2011). In contrast, pharmacological blockade of insulin signaling enhances olfactory sensitivity, even in animals in a fed state. These findings are consistent with the notion that insulin signaling promotes satiety. Insulin may function as a global metabolic signal in the fruit fly to suppress appetitive behaviors. Reduced levels of insulin in hunger state launch the expression of sNPFR1 that induces retuning of the local olfactory circuit (Figure 3D).

Underlying neural circuit for appetitive behaviors—In mammals, appetite is controlled by multiple brain regions (Berthoud, 2002). It has been well established that the hypothalamus integrates hormonal signals such as ghrelin, insulin and leptin from the gut, pancreas and adipose tissues, respectively. Subsequent activation of neurons containing NPY and AgRP in the arcuate nucleus of the hypothalamus augment food intake (Barsh and Schwartz, 2002; Magni et al., 2009). While important inroads have been made in identifying neuropeptides that regulate feeding behavior, little is understood about whether or how these hormones/neuropeptides alter olfaction and how that leads to behavioral changes. In rodents, internal state does influence olfactory responses in the olfactory cortex (Murakami et al., 2005) and a variety of different neuromodulators influence neural activity in the olfactory bulb (Shepherd, 2004). However, it is not clear whether these metabolic hormones are centrally read out in the olfactory cortex or whether they have more complicated interactions in different neural systems of the brain. A recent study in *Drosophila* suggests that internal metabolic states can modulate olfactory representation in the periphery (Root et al., 2011). A local signal by sNPF and a global metabolic cue by insulin are integrated at specific ORNs to change olfactory sensitivity (Figure 3D).

4. Concluding remarks

Features of the peripheral olfactory system are very similar between *Drosophila* and mammals. Although the odorant receptor gene families evolved independently, each receptor neuron expresses only one or a few receptor genes. Receptor neurons expressing the same gene converge onto the same glomerulus in both mice and *Drosophila*. Studies of neuromodulation in the last few years have discovered that other features are also shared between *Drosophila* and mammals. In both groups, GABA_B receptor-mediated presynaptic inhibition provides a gain control mechanism to modulate olfactory sensitivity. Furthermore, serotonin fine tunes this feedback inhibition by controlling the excitability of the local interneurons in *Drosophila* and periglomerular cells in mice. As these key sites of neuromodulation are conserved, it will be of great interest to learn whether the roles of NPY and tachykinins in reshaping olfactory sensitivity in the mammalian olfactory system are also conserved.

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Figure 1. Presynaptic inhibition mediated by GABA signaling in *Drosophila* antennal lobe
(A) Removing lateral input increases spike activity in projection neurons. Whole cell patch clamp was performed at the VM7 PNs. PN spike activity was shown with (magenta) and without (blue) lateral input from antennal glomeruli. VM7 ORN response (green) was shown as comparison. **(B)** GABA receptor antagonists mimic removal of lateral input to a projection neuron. **(C)** Activation of GABA_B receptors alters the gain of projection neurons. Two-photon microscopy was used to image PN calcium activity in flies expressing the calcium sensor G-CaMP in PNs. Graphs show the input-output function of PNs in saline (black circles) and in the presence of the GABA_BR antagonist CGP54626 (red squares). **(D)** Knockdown of GABA_B receptors impairs the ability of male flies in finding female flies. Images in **A** and **B** modified, with permission, from Olsen and Wilson, 2008. Images in **C** and **D** modified, with permission, from Root et al., 2008.

Figure 2. Serotonergic control of olfactory input and response of the secondary olfactory neurons

(A) Schematic diagram of the anatomical organization of the mammalian olfactory bulb. Neurotransmission from ORN axon (green) was monitored with spH and calcium levels of periglomerular cells (orange) was also measured in the experiments by Petzold et al., 2009. Modified with permission from (Dugue and Mainen, 2009). (B) WAY161503, an selective 5-HT_{2C} receptor agonist, increases olfactory response of the periglomerular cells, but attenuates the neurotransmitter release from ORNs in mice. Images modified, with permission, from Petzold et al., 2009. (C) Serotonin enhances the responses of GABAergic local interneurons to electrical stimulation of the antennal nerve in *Drosophila*. (D)

Differential enhancement of projection neuron responses by serotonin in *Drosophila*. Images in C and D modified, with permission, from Dacks et al., 2009.

Figure 3. Olfactory representation in projection neurons is altered by starvation in *Drosophila* (A) Two-photon imaging of PN calcium activity in response to apple cider vinegar. (B) Representative traces of fluorescence change over time for the five glomeruli excited by vinegar. (C) Peak response across a range of vinegar concentrations for each glomerulus. (D) A model for the cellular and molecular mechanism of starvation modulation of olfactory representation in the antennal lobe of *Drosophila*. Images modified, with permission, from Root et al., 2011