Chapter

Neuronal Architecture and Functional Organization of Olfactory Glomeruli

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Abstract

In the antennal lobes of insects and olfactory bulbs of vertebrates, the primary processing of olfactory information occurs within specialized units, called glomeruli. Glomeruli are discrete areas of densely packed, fine neuropil, usually ensheathed in glia cells. Glomeruli are the sites of synaptic interaction between axons of olfactory receptor cells and dendrites of central olfactory neurons. This chapter reviews the functional significance of this neuronal architecture, the glomerulus, with particular emphasis on results obtained in the sphinx moth, Manduca sexta. How is neuronal circuitry of olfactory glomeruli functionally organized, what attributes of olfactory stimuli are analyzed in glomeruli and how are these attributes processed and encoded in them? Glomeruli have been found in different invertebrate groups, such as crustaceans and insects with the glomeruli in the antennal lobes and the deutocerebrum, and molluscs with subepithelial glomeruli in the tentacle, as well as in different vertebrate groups such as amphibians, birds, fish, and mammals with glomeruli in the olfactory bulb. The organization of primary olfactory centers into glomeruli in diverse species suggests that glomeruli have a common and fundamental function in the processing of information about chemosensory stimuli and that glomeruli across taxa may share similar means of processing olfactory input.

Keywords: antenna, behavior, brain module, CNS, insect, *Manduca sexta*, neural coding, olfaction, orientation, pheromone, smell, synaptic integration

1. Introduction

Glomeruli in the brains of insects and vertebrates are the morphological and physiological structures where the primary processing of olfactory information takes place [1]. Glomeruli are housed in the olfactory centers of insects, the antennal lobes, and in the olfactory bulbs of vertebrates. Their widespread presence in different taxa has been interpreted to suggest common functionality. Experimental evidence based on recordings from principal output neurons in olfactory glomeruli of vertebrates and invertebrates supports this notion [2, 3]. The striking structural similarity, as well as the similarity of the responses to odor stimulation between neurons in the insect

antennal lobe and vertebrate olfactory bulb, suggests that glomerular microcircuits across taxa may share similar means of processing olfactory input [1, 4, 5]. Studies on glomerular circuitry address the central question of the functional organization of olfactory glomeruli.

The antennal lobes of the sphinx moth *M. sexta* have emerged as a model system to determine mechanisms underlying olfactory information processing in early olfactory centers such as the glomerular microcircuits. (1) In *M. sexta*, the antennal lobes house a male-specific olfactory subsystem. This subsystem is specialized to process information about the female sex pheromone [6]. Input and output relationships in this experimentally advantageous model system can be precisely defined. (2) Other glomeruli in the antennal lobes of *M. sexta* are clearly different in both function and morphology compared to the male-specific subsystem that comprises the macroglomerular complex (MGC). The MGC consists of three glomeruli, the toroid-1, toroid-2, and the cumulus [7, 8]. (3) The MGC receives input from antennal sensory neurons [9] that are specifically tuned to one of the two essential components of the female sex pheromone [10].

The glomeruli of the MGC process information about the two essential pheromone components of the female sex pheromone. The components of the odor stimulus released by the female have been determined in terms of the concentration and ratio of the pheromone components. The number of neurons projecting from the MGC to higher brain centers is relatively limited. About 30 to 40 projection neurons (PNs) innervate the MGC, and about 860 PNs innervate all the glomeruli in the AL [11]. Many local interneurons (LNs) and PNs in the ALs have been described both morphologically and physiologically [3, 8, 12, 13].

The goal of research on olfactory glomeruli is to understand the role(s) of individual glomeruli, for example, the glomeruli that constitute the MGC in olfaction, namely, the toroid-1, toroid-2, and the cumulus, by analyzing how the neural circuits associated with these glomeruli process pheromonal information. The functional organization of the MGC can be studied by means of single-unit intracellular recording, staining and laser scanning confocal microscopy, and more recently, imaging techniques, multi-unit recordings, and computational models [14–22]. This line of research attempts to address several topics: How do features of the stimulus determine pheromone-evoked response characteristics of MGC interneurons? How do MGC interneurons discern pheromone components in a complex odor blend? Can MGC–PNs resolve and encode the naturally intermittent temporal structure of pheromonal stimuli? Do the two essential pheromone components serve specific and different roles? Answers to these questions will help define the functional role of glomeruli in olfaction and will aid our understanding of how different features of an odor stimulus are processed in the brain.

2. The chemical senses

The chemical senses are the oldest senses. The earliest living organisms monitored their environment with chemoreception in order to sense the availability of nutrients [23, 24] and thus to respond to different chemicals. Higher organisms face the challenge of reacting to various internal and external chemicals, for example, hormones, neurotransmitters, neural recognition molecules, and intra- and interspecific olfactory, and gustatory signals [24, 25].

The olfactory pathway starts with peripheral structures. In the case of vertebrates, olfactory receptor cells are located in the nasal cavity in the olfactory epithelium. In insects, sensilla on the antennae of insects houses olfactory receptor cells [26]. Two areas in olfactory research have been under heavy investigation: (a) the transduction mechanisms taking place at the olfactory receptor cells and (b) the synaptic mechanisms acting at the first synaptic relay in the olfactory pathway, including synaptic plasticity and learning, that is, in the olfactory bulb (OB) of vertebrates and the antennal lobes (ALs) of insects [1, 27–30].

3. The glomerulus in olfaction

The structural unit of organization in the AL or OB is the glomerulus [24, 31–37], that is, the neuropil is arranged into discrete areas ensheathed by a glial envelope [38, 39]. In *M. sexta*, glial cells play an important role in the sculpturing of glomeruli, since early removal of glial cells results in an absence of these subunits [38]. Glomeruli are the sites of synaptic interaction between primary olfactory axons and dendritic arborizations of central olfactory neurons [40]. Unlike that observed in vertebrates, evidence for moths and cockroaches suggests that in insects, few or no synaptic interactions take place in the neuropil outside the glomeruli [41, 42].

The brain and nervous system can be considered as arrangements of modular structures. Glomeruli are a prime example of such modular structures that are repeated in a specific brain region. It was Camillo Golgi (1874, cited in [34]) who first noted glomeruli. Since his early discovery, other modular structures have been described. Examples include columns, barrels, barreloids, and blobs [33, 34]. Considerable variation has been described for these modular structures in different species. In closely related species, one of them can lacks such an iterated module of brain organization but still achieves the same behavioral functions as the species that has them [34].

Glomeruli have also been found in the cerebellar cortex and the thalamic regions of vertebrates [25, 43]. Olfactory glomeruli have a long evolutionary history as they have been described in phylogenetically old animal groups. These groups include marine crustacea, fishes, onychophora, myriapoda, and mollusca. Glomeruli appeared before animals transitioned from marine to terrestrial life forms [25].

Glomeruli are not only structural modules but also functional units [33, 44]. 2-deoxyglucose (2-DG) studies in neonatal rat pups established a focal point in the dorsal part of the olfactory bulb, the modified glomerular complex. This is a small group of glomeruli involved in processing of suckling odor cues. In *Drosophila melanogaster*, 2-DG mapping of odor-induced neuronal activity in the ALs labeled distinct and histologically identified glomeruli [45, 46]. In insects, the macroglomerular complex has been established as the first central site where information about the female sex pheromone is processed [6, 47]. During odor stimulation of the rat olfactory epithelium, neighboring mitral/tufted cells, that is, the output neurons of the olfactory bulb that innervate the same glomerulus in the olfactory bulb, were frequently simultaneously excited or inhibited compared to cells that innervated different glomeruli [48].

The existing data indicates that glomeruli are functional units such that information about odorants is represented in a spatial manner among glomeruli. When the olfactory epithelium is stimulated with most odorants, the resulting responses in the

AL or olfactory bulb are spatial gradients or patterns of activity in more than one glomerulus [23, 45, 46, 49–51]. Three measures of neural activation (voltage-sensitive dyes, the 2-DG method, and *c-fos* expression) have revealed that in mammals, different odors elicit overlapping but distinctly different patterns of glomerular activity [51–54]. In the cockroach *Periplaneta americana*, stimulation with the female sex pheromone evokes responses in a very limited number of neurons and glomeruli, whereas general odorants result in responses in different output neurons representing more than 10 out of 130 glomeruli [55]. In *D. melanogaster*, stimulation with complex odors as well as with individual odors results in a spatial pattern of 2-DG activity in different specific subsets of antennal lobe glomeruli [45, 46].

A synthesis of the diffuse as well as specific aspects of the primary olfactory projections to central sites came from Ken Mori et al. [56, 57]. They characterized individual mitral/tufted cells based on the range of odor molecules effective in activating each cell. Individual mitral/tufted cells showed excitatory responses to groups of molecules with similar chemical structure [57]. Imamura et al. [56] developed a model for the activation of individual mitral/tufted cells by a range of odor molecules. In the model that takes into account work in different research groups, an olfactory sensory neuron expresses one or, at most, a few different types of receptor proteins. Subsequently, a neuron is activated by odor molecules with similar structure. The olfactory pathway is thought to work with a one cell-one receptor rule [58] such that a sensory cell expresses only one among hundreds of possible molecular receptors [59]. Neurons with the same or similar receptor proteins send one axon each to one or a few glomeruli and thus define glomerular function [60, 61]. The tuning specificity of the mitral/tufted cells thus reflects the specificity of the receptor protein [54, 56]. Recent studies have indicated that individual receptor probes hybridize to a small number of olfactory glomeruli. This suggests that axons of sensory neurons expressing the same olfactory receptor protein converge on only a small number of glomeruli [60, 61]. Together with the notion that individual mitral/tufted cells arborizing in single glomeruli have similar response specificities, the resulting picture is that each glomerulus appears to have a unique mixture of inputs [52]. This input, in turn, limits its odor specificity, also known as its molecular receptive range.

4. The antennal lobes of the Sphinx Moth *M. sexta*

The insect antenna consists of three segments, namely, the scape, pedicel, and flagellum. The entire length of the antenna has hairs or sensilla on its surface. On the first two segments, the sensilla houses mechanosensitive neurons. These project to mechanosensory centers in the deutocerebrum [62]. In the sphinx moth *M. sexta*, the long flagellum, divided into 85–90 annuli, is equipped with about $4x10^5$ sensilla. These represent several modalities, such as mechanosensation, hygroreception, and olfaction [10, 63–65]. The sensory neurons in olfactory and possibly other antennal sensilla send their axons to the antennal lobes (**Figure 1**). The sensory neurons converge onto central interneurons. In *P. americana*, the convergence ratio between olfactory sensory neurons and projection neurons can be as high as 5000 to 1, and in rabbits, the ratio between sensory neurons and mitral cells is about 1000 to1 [40, 67]. The antennal lobe of *M. sexta* contains about 64 spheroidal glomeruli [68, 69]. In male *M. sexta*, a macroglomerular complex located near the entrance of the antennal nerve into the antennal lobes has been identified (**Figure 1**) [6].

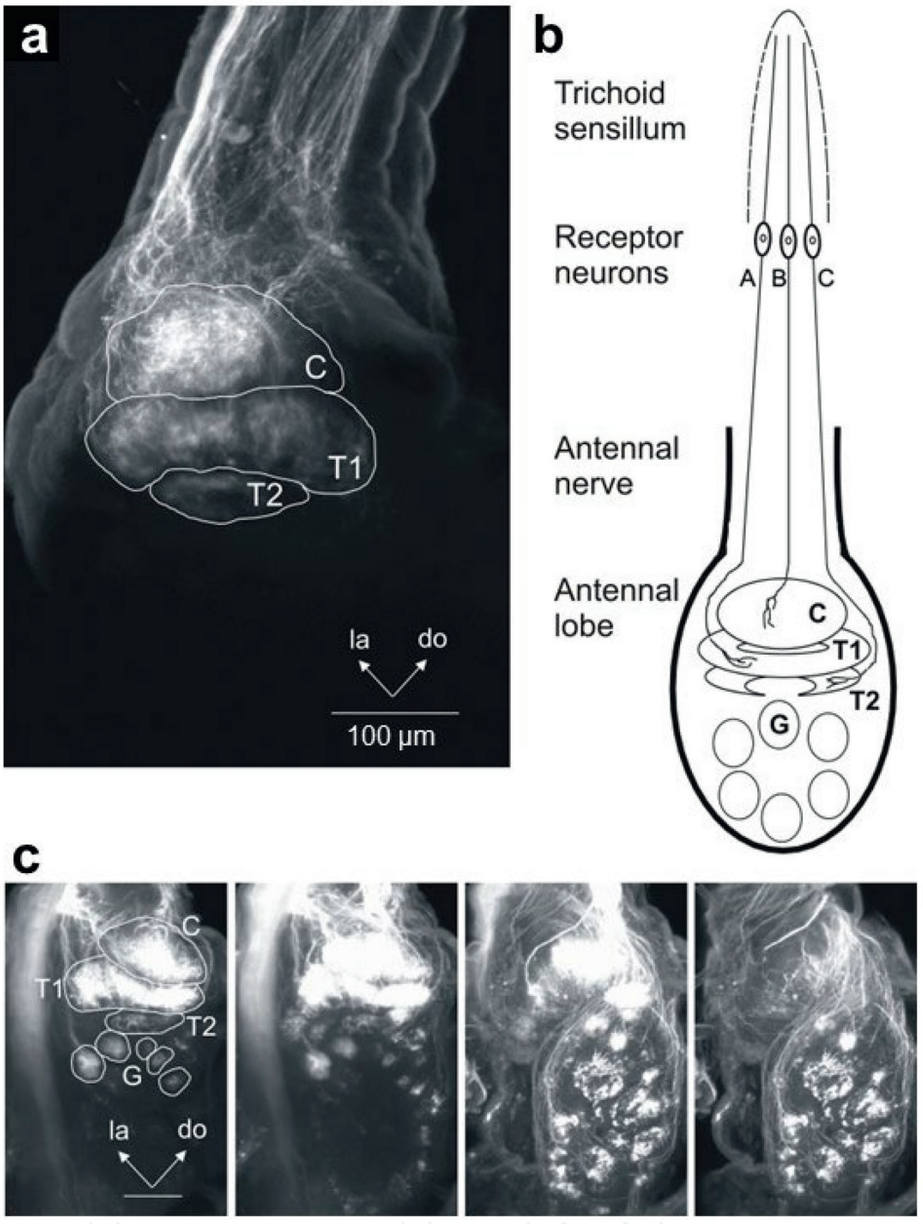

Figure 1.
This figure shows how antennal sensory neurons project to glomeruli in the antennal lobes of Manduca sexta. Sensory neurons were anterogradely labeled with rhodamine dextran. The diagram and images show frontal views. (a) Olfactory receptor neurons in long antennal trichoid sensilla project to the three glomeruli of the macroglomerular complex (MGC: C-cumulus, T1-toroid-1, and T2-toroid-2). (b) a schematic diagram illustrates how receptor neurons project to the antennal lobe. Receptor neurons from a trichoid sensillum project to the three glomeruli of the macroglomerular complex. (c) if receptor neurons in long trichoid sensilla and other antennal sensilla were labeled, axonal projections would go to the macroglomerular complex and ordinary glomeruli in the antennal lobes. Optical sections were taken at different depths in anterior to posterior direction through the antennal lobes shown from left to right. C – Cumulus, do – Dorsal, la – Lateral, T1 – Toroid-1, T2 – Toroid-2. Scale bar: 100 µm. From [66].

The first glomeruli in insects were described in the deutocerebrum of the bee by Kenyon [25, 70, 71]. In *M. sexta*, a closer anatomical analysis of glomeruli revealed a complex substructure of discrete domains and laminae within individual glomeruli [7, 72]. In bees, however, glomeruli have a relatively simple organization [73].

In contrast to the large differences in the number of glomeruli among different animal species, insect antennal systems present highly invariant glomerular organizations

with regard to shape, size, location, and number within a species [74]. This has been shown for a variety of species including the fruit fly *Drosophila melanogaster* [75, 76], sphinx moth *M. sexta* [68], moth *Mamestra brassicae* [31], bee *Apis mellifera* [77], and cockroach *Blaberus craniifer* [78]. This invariance was also found to be true for the iulid *Cylindroiulus punctatus* (Diplopoda) [79] and in a vertebrate, the zebrafish (*Brachydanio rerio*) [80]. The number of glomeruli in all of these species is relatively small (18 for *C. punctatus* to 174 in worker bees). It is more difficult to verify numerical invariance in vertebrates with several thousand glomeruli [55]. The only identified vertebrate glomerulus is the modified glomerular complex for detection of the maternal suckling pheromone in rats [81].

5. Morphology and immunocytochemistry of neurons in the antennal lobe

Three classes of interneurons are present in the antennal lobes [11, 82]: (1) local, amacrine interneurons (LNs), with arborizations limited to the antennal lobe; (2) projection neurons (PNs) that send axons to higher order brain centers; and (3) centrifugal neurons that send axons from higher order brain centers into the antennal lobe (**Figures 2** and **3**). Sensory neurons from the antenna send their axon into one glomerulus only [9, 62] where it forms synapses with LNs, presumably mediated by acetylcholine [83]. The somata of antennal lobe LNs and PNs form three groups (lateral, medial, and anterior) [82]. There are about 360 LNs in each antennal lobe of *M. sexta*. They can innervate many, and perhaps all, glomeruli and appear to be mostly GABAergic [84, 85]. Different neurophysiological categories of local interneurons have been observed with respect to patterns of postsynaptic activity [13]. Evidence for unidirectional synaptic interactions between local interneurons and projection neurons as well as for disinhibitory pathways between these two types of neurons was found [13]. About 860 PNs project axons out of the antennal lobe through various antenno-cerebral tracts to different parts of the protocerebrum, for example, the calyces of the mushroom body and the lateral horn of the protocerebrum [11]. The third group of neurons, centrifugal neurons, is small in number and consists of a variety of cell types with unique morphologies, some of which innervate all glomeruli of one or both antennal lobes [82, 86]. The antennal lobe possesses a single serotonin-immunoreactive neuron [86]. This neuron has its soma in one antennal lobe, which innervates all glomeruli in the contralateral antennal lobe where it forms and receives synapses and has arborizations in the ipsilateral and contralateral protocerebrum [87].

Acetylcholine and GABA are the most prominent neurotransmitters in the antennal lobe [83]. Evidence that acetylcholine may serve as a transmitter has been reported for antennal sensory neurons [88] and some classes of projection neurons [89]. Acetylcholine may be released by primary afferent axons synapsing onto AL neurons [88, 90–93]. GABA is prominent in local interneurons and is also present in a subset of PNs [84]. GABA has an important role in the synaptic inhibition of PNs [85]. An IPSP is evoked when the antenna is stimulated with an odor. The IPSP is mediated by a chloride conductance and is sensitive to reversible blockade by picrotoxin and bicuculline. GABA hyperpolarizes neurons and inhibits their spontaneous nerve impulse firing. Several antennal lobe neurons are immunoreactive for biogenic amines. These neurons have wide dendritic arborizations and are thought to have widespread effects. Possibly, these neurons mediate central modulation of synaptic activity or threshold levels within the antennal lobe [86].

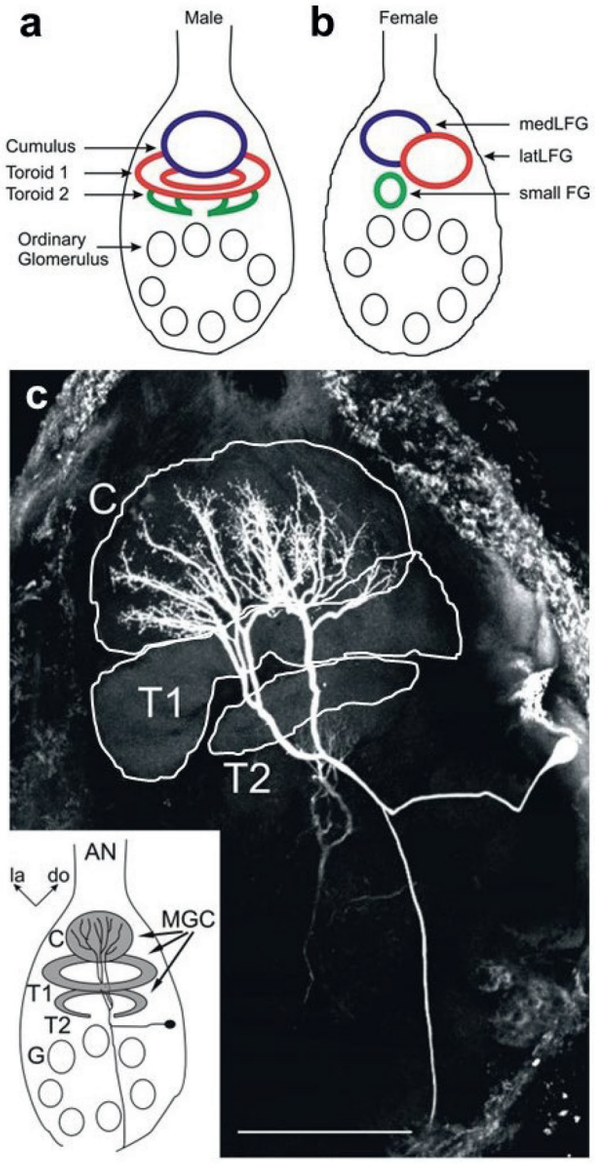

Figure 2.
Diagrammatic representation of sexually isomorphic glomeruli (G) and sexually dimorphic glomeruli in (a) male and (b) female Manduca sexta. (c) Laser-scanning confocal micrograph of an antennal lobe projection neuron in the moth antennal lobe of M. sexta. Image of a projection neuron in the macroglomerular complex (MGC-PN) with arborizations confined to the cumulus. The inset illustrates the organization of the antennal lobe with the macroglomerular complex (MGC) and other glomeruli (G). latLFG – Lateral large female glomerulus, medLFG – Medial large female glomerulus, smallFG – Small female glomerulus, C - cumulus, T1 - toroid-1, T2 - toroid-2, G – glomerulus, MGC – Macroglomerular complex, la – Lateral, do - dorsal. Scale bar: 100 μm. Modified from [66].

In LNs and PNs, several putative neuropeptides appear to be colocalized with classical transmitters [89].

6. The male-specific macroglomerular complex

In male *M. sexta*, the approximately 42,000 long trichoid sensilla commonly each contain two bipolar olfactory-sensory neurons that project to the macroglomerular complex (MGC) in the AL [9, 63–65]. Each of these two neurons is very sensitive

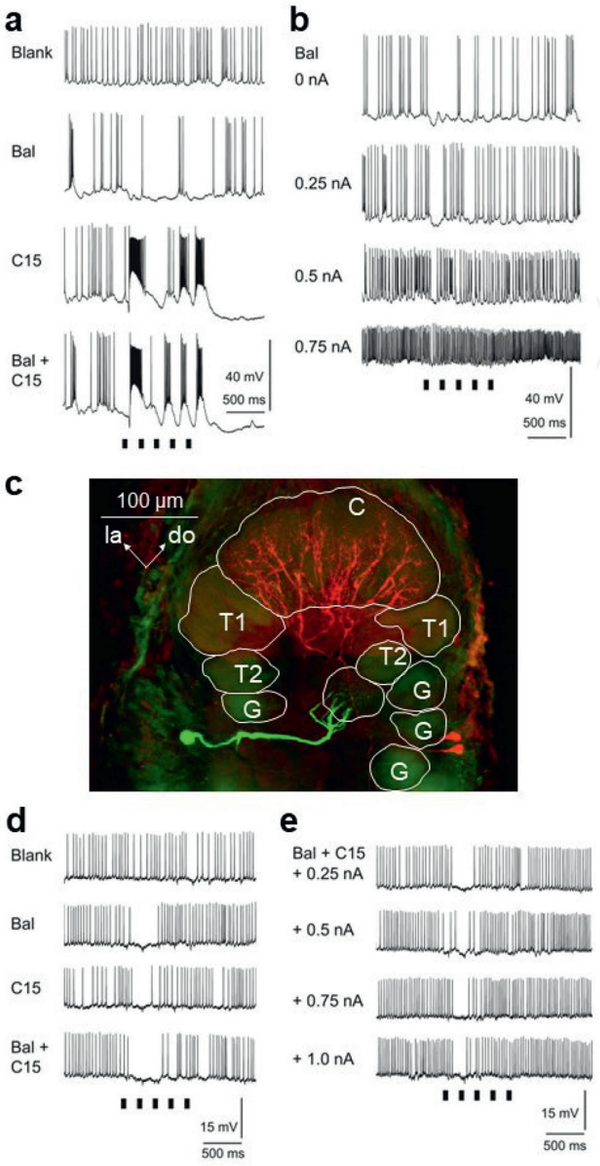

Figure 3.
The figure shows the intracellularly recorded physiological responses of labeled projection neurons in the antennal lobes of the sphinx moth Manduca sexta during antennal stimulation with pheromone. (a) this C15-specialist neuron responded with membrane potential inhibition to a stimulus that contained C15. This was followed by strong depolarization and again inhibition (third and fourth trace). Likewise, antennal stimulation with the pheromone blend of C15 + BAL evoked a mixed response. In contrast, stimulation with bombykal (BAL) evoked an inhibitory response. The antenna was stimulated with five 50-ms stimulus pulses at 5 Hz. The stimulus markers are depicted as black boxes beneath the records. (b) the membrane potential of the neuron was depolarized by injecting current through the recording electrode. As a result, the nerve impulse firing frequency increased, whereas the first stimulus pulse of BAL induced a membrane hyperpolarization and reduction in firing (inhibition). (c) the laser scanning confocal micrograph shows the morphology of two projection neurons in the antennal lobe. The neuron labeled in red, stained with biocytin, is described in figure panels (a) and (b), whereas panels (d) and (e) show the responses of the green, uniglomerular projection neuron, which is in Lucifer yellow (frontal view). C15-specialist neuron, the red neuron, has dendritic branches in the cumulus glomerulus of the macroglomerular complex and not in the toroid-1 or any other glomerulus. The green neuron sent dendritic branches into only one ordinary glomerulus. (d) Electrophysiological recordings from an antennal lobe projection neuron that innervated one of the ordinary glomeruli. When the antenna was stimulated with bomybkal, C15, or a blend of both pheromone components, the neuron responded with a reduction of the firing rate. As in panels (a) and (b), five identical stimulus pulses were delivered to the ipsilateral antenna at a frequency of 5 Hz. (e) Antennal stimulation of the same neuron with the pheromone blend resulted in inhibition, even when the membrane potential of the neuron was depolarized through current injection. The first antennal pheromone stimulus resulted in membrane hyperpolarization. C - cumulus; do - dorsal; G - ordinary glomerulus; la - lateral; me - medial; T1 - toroid-1; T2 - toroid-2. Scale bar = 100 µm. From [66].

to stimulation with one of the two major female sex-pheromone components, bombykal ((E,Z)-10,12-hexadecadienal) and a hexadecatrienal ((E,E,Z)-10,12,14-hexadecatrienal) [10]; that is, they have narrow molecular receptive ranges and constitute highly specific input channels.

In addition to the 64 spheroidal, ordinary glomeruli, the antennal lobe of *M. sexta* houses the sexually dimorphic MGC [69]. The MGC consists of at least three glomeruli (**Figures 2** and **3**) [7, 8, 16, 66]. One is donut-shaped (the "toroid-1"), and the other has a more globular structure (the "cumulus"). The third one (the toroid-2) is of unknown function and appears to have a donut shape as well. The cumulus is situated on the toroid and closer to the entrance of the antennal nerve. Projection neurons (PNs) with arborizations in the toroid-1 respond preferentially to antennal stimulation with the principal pheromone component bombykal (Bal-specialist MGC-PNs), whereas PNs arborizing in the cumulus respond preferentially to the second key

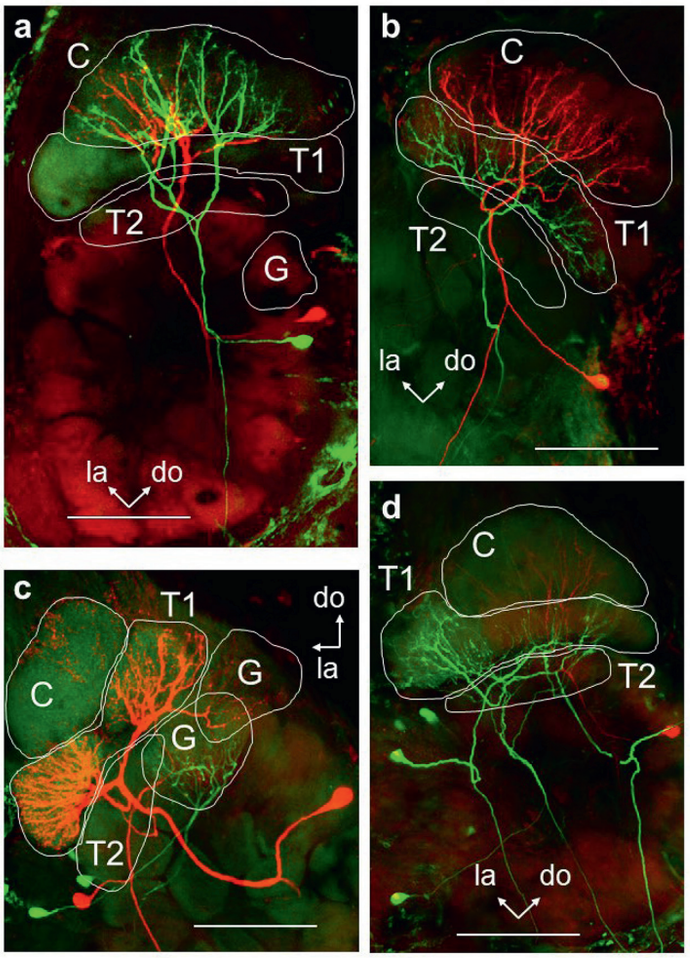

Figure 4.
Laser scanning confocal images illustrating the morphological diversity of projection neurons in the antennal lobes of the sphinx moth Manduca sexta. (a) Two C15 (E,Z-11,13-pentadecadienal) -specialist MGC-PNs (projection neurons of the macroglomerular complex) with arborizations confined to the cumulus. While the branches of the two neurons apparently overlapped in certain parts of the cumulus (indicated in yellow), other parts were innervated by just one of the two neurons. (b) One C15 specialist MGC-PN arborizing in the cumulus (green), and one bombykal (Bal) -specialist MGC-PN arborizing in the toroid-1 (red). (c) One bombykal (Bal) -specialist MGC-PN arborizing in the toroid-1 (red), and two projection neurons (red and green) innervating ordinary glomeruli adjacent to the MGC. (d) Several MGC-PNs innervating either the cumulus (red) or the toroid-1 (green). C – Cumulus, T1 – Toroid-1, T2 – Toroid-2, G – Ordinary glomerulus, do – Dorsal, la - lateral. Scale bar: 100 μm. Modified from [16].

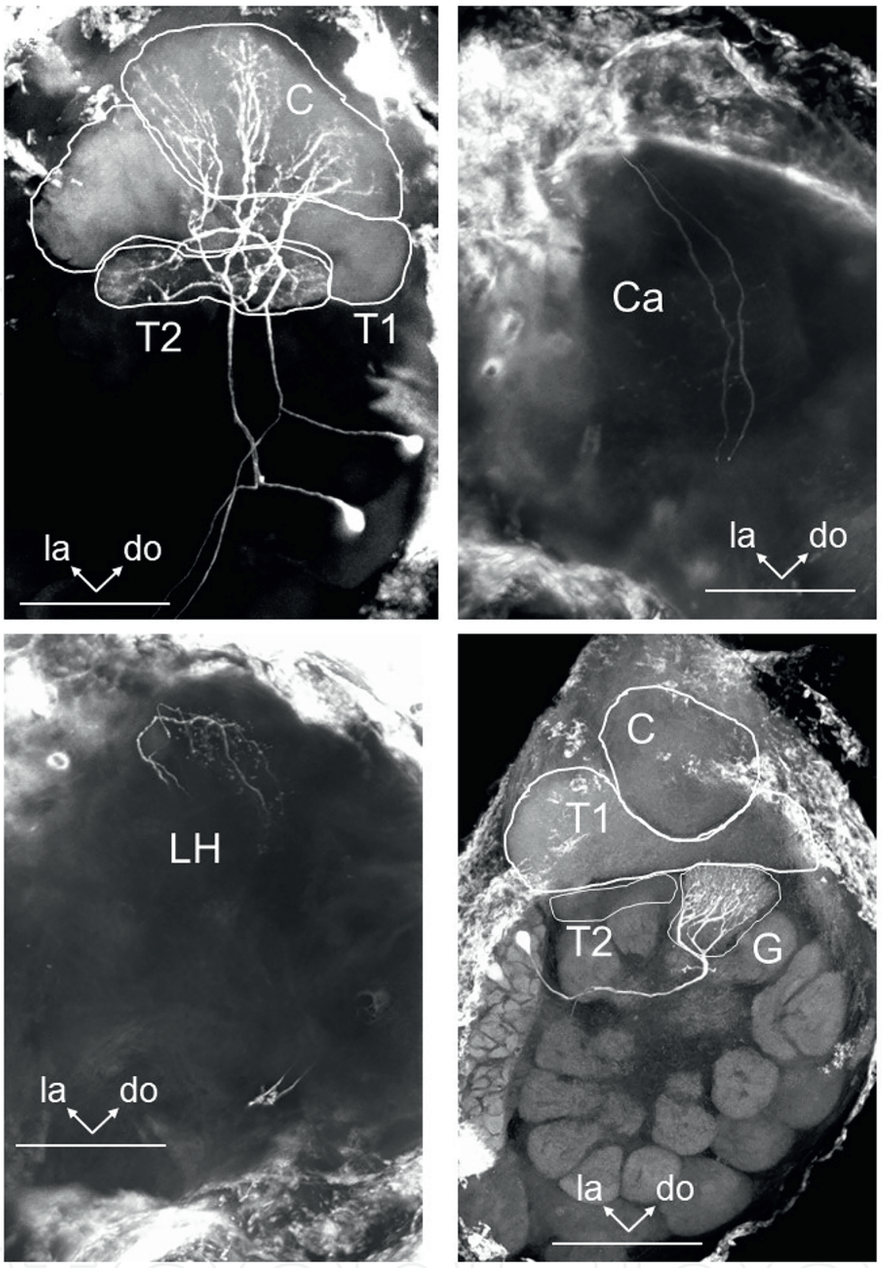

Figure 5.
Morphology (frontal view) of antennal lobe projection neurons. (a) a C15-specialist MGC-PN sent dendrites into the cumulus, while the branches of another MGC-PN were confined to the toroid-2. (b) the axons of the neurons shown in (a) left the antennal lobe and projected via the inner antenno-cerebral tract to the ipsilateral protocerebrum where they sent collaterals into the calyces (Ca) of the mushroom body and (c) terminated in the lateral horn (LH). (d) another PN showed branches in an ordinary glomerulus adjacent to the MGC. Do - dorsal, la - lateral, C – Cumulus, G – Ordinary glomerulus, T1 – Toroid-1, T2 -toroid-2. Scale bar: 100 μm.

component of the pheromone, a hexadecatrienal [8]. These neurons also respond to E,Z-11,13-pentadecadienal (C15), a chemically more stable mimic of the second key component of the sex pheromone E,E,Z-10,12,14-hexadecatrienal [10, 94] and are referred to as C15-specialist MGC-PNs.

Many AL neurons in *M. sexta* have been characterized morphologically and physiologically (**Figures 3–5**) [3, 4, 6, 11, 12, 85]. Neurophysiological studies of the pheromone-specific olfactory subsystem in male moths have focused on three properties of the sex-pheromone stimulus and on how these properties affect the central processing of sex-pheromone information [4, 12, 83]. The properties are the quality

or chemical composition of the pheromone blend, the quantity or concentrations of individual pheromone components, and the temporal structure of the stimulus or its intermittency. Odor stimuli such as pheromones exist in wind plumes in the form of filaments and blobs of different concentration.

7. Conclusions

An important issue in the organization and operation of the insect olfactory system is the functional significance of glomeruli in the antennal lobes. Research on the sphinx moth *Manduca sexta* has provided a firm foundation of technical experience and knowledge about an experimentally favorable model system that allows the study of glomerular structure and function with greater precision than has been possible in other species [95–97]. Specifically, glomeruli in the olfactory subsystem of male *M. sexta*, which are designated for pheromone processing with its anatomically and functionally identified, male-specific neuropil, contribute to our understanding of the neuronal architecture and functional organization of olfactory glomeruli.

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Conflict of interest

The author declares no conflict of interest.

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References

[1] Shepherd GM. Neurobiology, modules for molecules. Nature. 1992;**358**(6386):457-458

[2] Hamilton KA, Kauer JS. Intracellular potentials of salamander mitral/tufted neurons in response to odor stimulation. Brain Research. 1985;**338**(1):181-185

[3] Christensen TA, Hildebrand JG. Male-specific, sex pheromone-selective projection neurons in the antennal lobes of the moth *Manduca sexta*. Journal of Comparative Physiology. A. 1987;**160**(5):553-569

[4] Christensen TA, Heinbockel T, Hildebrand JG. Olfactory information processing in the brain: Encoding chemical and temporal features of odors. Journal of Neurobiology. 1996;**30**(1):82-91

[5] Hildebrand JG, Shepherd GM. Mechanisms of olfactory discrimination: Converging evidence for common principles across phyla. Annual Review of Neuroscience. 1997;**20**:595-631

[6] Matsumoto SG, Hildebrand JG. Olfactory mechanisms in the moth *Manduca sexta*: Response characteristics and morphology of central neurons in the antennal lobes. Proceedings of the Royal Society of London B. 1981;**213**:249-277

[7] Strausfeld NJ. Cellular organization in male-specific olfactory neuropil in the moth *Manduca sexta*. In: Elsner N, Singer W, editors. Dynamics and Plasticity in Neuronal Systems. Stuttgart, Germany: Thieme; 1989. p. 79

[8] Hansson BS, Christensen TA, Hildebrand JG. Functionally distinct subdivisons of the macroglomerular complex in the antennal lobe of the male sphinx moth *Manduca sexta*. The Journal of Comparative Neurology. 1991;**312**:264-278

[9] Christensen TA, Harrow ID, Cuzzocrea C, Randolph PW, Hildebrand JG. Distinct projections of two populations of olfactory receptor neurons in the antennal lobe of the sphinx moth *Manduca sexta*. Chemical Senses. 1995;**20**:313-323

[10] Kaissling K-E, Hildebrand JG, Tumlinson JH. Pheromone receptor cells in the male moth *Manduca sexta*. Archives of Insect Biochemistry and Physiology. 1989;**10**:273-279

[11] Homberg U, Christensen TA, Hildebrand TA. Structure and function of the deutocerebrum in insects. Annual Review of Entomology. 1989;**34**:477-501

[12] Kanzaki R, Arbas EA, Strausfeld NJ, Hildebrand JG. Physiology and morphology of projection neurons in the antennal lobe of the male moth *Manduca sexta*. Journal of Comparative Physiology. A. 1989;**165**:427±453

[13] Christensen TA, Waldrop B, Harrow ID, Hildebrand JG. Local interneurons and information processing in the olfactory glomeruli in the moth *Manduca sexta*. Journal of Comparative Physiology. A. 1993;**173**:385-399

[14] Heinbockel T, Hildebrand JG. Antennal receptive fields of pheromone-responsive projection neurons in the antennal lobes of the male sphinx moth *Manduca sexta*. Journal of Comparative Physiology. A. 1998;**183**(2):121-133

[15] Heinbockel T, Kloppenburg P, Hildebrand JG. Pheromone-evoked

potentials and oscillations in the antennal lobes of the sphinx moth *Manduca sexta*. Journal of Comparative Physiology. A. 1998;**182**(6):703-714

[16] Heinbockel T, Christensen TA, Hildebrand JG. Temporal tuning of odor responses in pheromone-responsive projection neurons in the brain of the sphinx moth *Manduca sexta*. The Journal of Comparative Neurology. 1999;**409**(1):1-12

[17] Heinbockel T, Christensen TA, Hildebrand JG. Representation of binary pheromone blends by glomerulus-specific olfactory projection neurons. Journal of Comparative Physiology. A, Neuroethology, Sensory, Neural, and Behavioral Physiology. 2004;**190**(12):1023-1037

[18] Reisenman CE, Heinbockel T, Hildebrand JG. Inhibitory interactions among olfactory glomeruli do not necessarily reflect spatial proximity. Journal of Neurophysiology. 2008;**100**(2):554-564

[19] Christensen TA, Pawlowski VM, Lei H, Hildebrand JG. Multi-unit recordings reveal context-dependent modulation of synchrony in odor-specific neural ensembles. Nature Neuroscience. 2000;**3**(9):927-931

[20] Delahunt CB, Riffell JA, Kutz JN. Biological mechanisms for learning: A computational model of olfactory learning in the *Manduca sexta* moth, with applications to neural nets. Frontiers in Computational Neuroscience. 2018;**19**:102

[21] Martin JP, Lei H, Riffell JA, Hildebrand JG. Synchronous firing of antennal-lobe projection neurons encodes the behaviorally effective ratio of sex-pheromone components in male *Manduca sexta*. Journal of Compound Physiology A. 2013;**199**(11):963-979

[22] Lei H, Reisenman CE, Wilson CH, Gabbur P, Hildebrand JG. Spiking patterns and their functional implications in the antennal lobe of the tobacco hornworm *Manduca sexta*. PLoS One. 2011;**6**(8):e23382

[23] Shepherd GM. Principles of specificity and redundancy underlying the organization of the olfactory system. Microscopy Research and Technique. 1993;**24**(2):106-112

[24] Hildebrand JG. Analysis of chemical signals by nervous systems. Proceedings of the National Academy of Sciences of the United States of America. 1995;**92**(1):67-74

[25] Dethier VG. Five hundred million years of olfaction. In: Colbow K, Allison F, Linvilles RH, editors. Wright Lectures on Olfactory Research. Burnaby, B.C., Canada: Simon Fraser University; 1990. pp. 1-37

[26] Carr WE, Gleeson RA, Trapido-Rosenthal HG. The role of perireceptor events in chemosensory processes. Trends in Neurosciences. 1990;**13**(6):212-215

[27] Anton S, Rössler W. Plasticity and modulation of olfactory circuits in insects. Cell and Tissue Research. 2021;**383**:149-164

[28] Braubach O, Croll RP. The glomerular network of the zebrafish olfactory bulb. Cell and Tissue Research. 2021;**383**:255-271

[29] Marachlian E, Klappenbach M, Locatelli F. Learning-dependent plasticity in the antennal lobe improves discrimination and recognition of odors in the honeybee. Cell and Tissue Research. 2021;**383**:165-175

[30] Mori K, Sakano H. Olfactory circuitry and Behavioral decisions.

Annual Review of Physiology. 2021;**83**:231-256

[31] Rospars JP. Invariance and sex-specific variations of the glomerular organization in the antennal lobes of a moth, Mamestra brassicae, and a butterfly, Pieris brassicae. Journal of Compound Neurology. 1983;**220**(1):80-96

[32] Strausfeld NJ. Insect vision and olfaction: Common design principles of neuronal organization. In: Singh RN, Strausfeld NJ, editors. Neurobiology of Sensory Systems. Boston, MA: Springer; 1989a

[33] Shepherd GM. Contribution toward a theory of olfaction. In: Colbow K, Allison F, Linvilles RH, editors. Wright Lectures on Olfactory Research. Simon Fraser University; 1990. pp. 61-109

[34] Purves D. Neural Activity and the Growth of the Brain. Cambridge: Cambridge University Press; 1994

[35] Fuscà D, Kloppenburg P. Odor processing in the cockroach antennal lobe—The network components. Cell and Tissue Research. 2021;**383**:59-73

[36] Paoli M, Galizia GC. Olfactory coding in honeybees. Cell and Tissue Research. 2021;**383**:35-58

[37] Wheelwright M, Whittle CR, Riabinina O. Olfactory systems across mosquito species. Cell and Tissue Research. 2021;**383**:75-90

[38] Tolbert LP, Oland LA. A role for glia in the development of organized neuropilar structures. Trends in Neurosciences. 1989;**12**(2):70-75

[39] Tolbert LP. Intercellular interactions in the constructions of olfactory glomeruli in an insect. In: Døving KB, editor. Proc Xth International Symposium on Olfaction and Taste (ISOT X). Oslo, Norway: University of Oslo; 1990. pp. 236-245

[40] Shepherd GW, Chen WR, Greer CA. Olfactory bulb. In: Shepherd GM, editor. The Synaptic Organization of the Brain. Oxford: New York, NY; 2004. pp. 165-216

[41] Tolbert LP, Hildebrand JG. Organization and synaptic ultrastructure of glomeruli in the antennal lobes of the moth*Manduca sexta*: A study using thin sections and freeze-fracture. Proceedings of the Royal Society London. 1981;**24**(3):213-279

[42] Boeckh J, Tolbert LP. Synaptic organization and development of the antennal lobe in insects. Microscopy Research and Technique. 1993;**24**(3):260-280

[43] Heimer L. The Human Brain and Spinal Cord. New York: Springer; 1983. p. 402

[44] Lodovichi C. Topographic organization in the olfactory bulb. Cell Tissue Research. 2021;**383**(1):457-472

[45] Rodrigues V, Buchner E. [3H]2-deoxyglucose mapping of odor-induced neuronal activity in the antennal lobes of Drosophila melanogaster. Brain Research. 1984;**324**(2):374-378

[46] Rodrigues V. Spatial coding of olfactory information in the antennal lobe of Drosophila melanogaster. Brain Research. 1988;**453**(1-2):299-307

[47] Boeckh J, Boeckh V. Threshold and odor specificity of pheromone-sensitive neurons in the deutocerebrum ofAntheraea pernyi andA. polyphemus (Saturnidae). Journal of Comparative Physiology. 1979;**132**:235-242

[48] Buonviso N, Chaput MA. Response similarity to odors in olfactory bulb output cells presumed to be connected to the same glomerulus: Electrophysiological study using simultaneous single-unit recordings. Journal of Neurophysiology. 1990;**63**(3):447-454

[49] Kauer JS. Contributions of topography and parallel processing to odor coding in the vertebrate olfactory pathway. Trends in Neurosciences. 1991;**14**(2):79-85

[50] Scott JW. Central processing of olfaction. The Journal of Steroid Biochemistry and Molecular Biology. 1991;**39**(4B):593-600

[51] Kauer JS, Cinelli AR. Are there structural and functional modules in the vertebrate olfactory bulb? Microscopy Research and Technique. 1993;**24**(2):157-167

[52] Shepherd GM, Firestein S. Making scents of olfactory transduction. Current Biology. 1991;**1**(4):204-206

[53] Guthrie KM, Anderson AJ, Leon M, Gall C. Odor-induced increases in c-fos mRNA expression reveal an anatomical "unit" for odor processing in olfactory bulb. Proceedings of the National Academy of Sciences of the United States of America. 1993;**90**(8):3329-3333

[54] Mori K, Shepherd GM. Emerging principles of molecular signal processing by mitral/tufted cells in the olfactory bulb. Seminars in Cell Biology. 1994;**5**(1):65-74

[55] Boeckh J, Distler P, Ernst KD, Hösl M, Malun D. Olfactory bulb and antennal lobe. In: Schild D, editor. Chemosensory Information Processing. Berlin, Heidelberg: Springer; 1990. pp. 201-228

[56] Imamura K, Mataga N, Mori K. Coding of odor molecules by mitral/tufted cells in rabbit olfactory bulb. I. Aliphatic compounds. Journal of Neurophysiology. 1992;**68**(6):1986-2002

[57] Katoh K, Koshimoto H, Tani A, Mori K. Coding of odor molecules by mitral/tufted cells in rabbit olfactory bulb.II. Aromatic compounds. Journal of Neurophysiology. 1993;**70**(5):2161-2175

[58] Lancet D. Olfaction. Exclusive Receptors. Nature. 1994;**372**(6504): 321-322

[59] Chess A, Simon I, Cedar H, Axel R. Allelic inactivation regulates olfactory receptor gene expression. Cell. 1994;**78**(5):823-834

[60] Ressler KJ, Sullivan SL, Buck LB. Information coding in the olfactory system: Evidence for a stereotyped and highly organized epitope map in the olfactory bulb. Cell. 1994;**79**(7):1245-1255

[61] Vassar R, Chao SK, Sitcheran R, Nuñez JM, Vosshall LB, Axel R. Topographic organization of sensory projections to the olfactory bulb. Cell. 1994;**79**(6):981-991

[62] Kloppenburg P, Camazine SM, Sun XJ, Randolph P, Hildebrand JG. Organization of the antennal motor system in the sphinx moth *Manduca sexta*. Cell and Tissue Research. 1997;**287**(2):425-433

[63] Sanes JR, Hildebrand JG. Structure and development of antennae in a moth,*Manduca sexta*. Developmental Biology. 1976;**51**(2):280-299

[64] Keil TA. Fine structure of the pheromone-sensitive sensilla on the antenna of the hawkmoth, Manduca sexta. Tissue Cell. 1989;**21**(1):139-151

[65] Lee JK, Strausfeld NJ. Structure, distribution and number of surface sensilla and their receptor cells on the olfactory appendage of the male moth *Manduca sexta*. Journal of Neurocytology. 1990;**19**(4):519-538

[66] Heinbockel T, Shields VD, Reisenman CE. Glomerular interactions in olfactory processing channels of the antennal lobes. Journal of Compound Physiology A. 2013;**99**(11):929-946

[67] Boeckh J, Ernst KD, Sass H, Waldow U. Anatomical and physiological characteristics of individual neurones in the central antennal pathway of insects. Journal of Insect Physiology. 1984;**30**(1):15-26

[68] Rospars JP, Hildebrand JG. Anatomical identification of glomeruli in the antennal lobes of the male sphinx moth *Manduca sexta*. Cell and Tissue Research. 1992;**270**(2):205-227

[69] Rospars JP, Hildebrand JG. Sexually dimorphic and isomorphic glomeruli in the antennal lobes of the sphinx moth *Manduca sexta*. Chemical Senses. 2000;**25**(2):119-129

[70] Kenyon FC. The brain of the bee. A preliminary contribution to the morphology of the nervous system of the arthropoda. The Journal of Comparative Neurology. 1896;**6**:133-210

[71] Ernst KD, Boeckh J, Boeckh V. A neuroanatomical study on the organization of the central antennal pathways in insects. Cell and Tissue Research. 1977;**176**(3):285-306

[72] Strausfeld NJ. Selective staining reveals complex microstructure within antennal lobe glomeruli of *Manduca sexta*.Cellular organization in male-specific olfactory neuropil in the moth *Manduca sexta*. In: Elsner N, Barth FG, editors. Sense organs: interface between environment and behavior. Stuttgart, Germany: Thieme; 1988. p. 67

[73] Masson C, Mustaparta H. Chemical information processing in the olfactory system of insects. Physiological Reviews. 1990;**70**(1):199-245

[74] Rospars JP, Chambille I. Deutocerebrum of the cockroach Blaberus craniifer Burm. Quantitative study and automated identification of the glomeruli. Journal of Neurobiology. 1981;**12**(3):221-247

[75] Stocker RF. The organization of the chemosensory system in Drosophila melanogaster: A review. Cell and Tissue Research. 1994;**275**(1):3-26

[76] Stocker RF, Lienhard MC, Borst A, Fischbach KF. Neuronal architecture of the antennal lobe in Drosophila melanogaster. Cell and Tissue Research. 1990;**262**(1):9-34

[77] Arnold G, Masson C, Budharugsa S. Comparative study of the antennal lobes and their afferent pathway in the worker bee and the drone (Apis mellifera). Cell and Tissue Research. 1985;**242**:593-605

[78] Chambille I, Masson C, Rospars JP. The deutocerebrum of the cockroach Blaberus craniifer Burm. Spatial organization of the sensory glomeruli. Journal of Neurobiology. 1980;**11**(2):135-157

[79] Duy-Jacquemin MN, Arnold G. Spatial organization of the antennal lobe in Cylindroiulus punctatus (leach) (Myriapoda: Diplopoda). International Journal of Insect Morphology and Embryology. 1991;**20**(4-5):205-214

[80] Baier H, Korsching S. Olfactory glomeruli in the zebrafish form an invariant pattern and are identifiable

across animals. The Journal of Neuroscience. 1994;**14**(1):219-230

[81] Teicher MH, Stewart WB, Kauer JS, Shepherd GM. Suckling pheromone stimulation of a modified glomerular region in the developing rat olfactory bulb revealed by the 2-deoxyglucose method. Brain Research. 1980;**194**(2):530-535

[82] Homberg U, Montague RA, Hildebrand JG. Anatomy of antenno-cerebral pathways in the brain of the sphinx moth *Manduca sexta*. Cell and Tissue Research. 1988;**254**(2):255-281

[83] Hildebrand JG, Christensen TA, Arbas EA, Hayashi JH, Homberg U, Kanzaki R, et al. Olfaction in *Manduca sexta*: Cellular mechanisms of responses to sex pheromone. In: Duce IR, editor. Proc NEUROTOX 91 – Molecular Basis of Drug & Pesticide Action. London: Elsevier Applied Science; 1992. pp. 323-338

[84] Hoskins SG, Homberg U, Kingan TG, Christensen TA, Hildebrand JG. Immunocytochemistry of GABA in the antennal lobes of the sphinx moth *Manduca sexta*. Cell and Tissue Research. 1986;**244**(2):243-252

[85] Waldrop B, Christensen TA, Hildebrand JG. GABA-mediated synaptic inhibition of projection neurons in the antennal lobes of the sphinx moth, Manduca sexta. Journal of Compound Physiology A. 1987;**161**(1):23-32

[86] Kent KS, Hoskins SG, Hildebrand JG. A novel serotonin-immunoreactive neuron in the antennal lobe of the sphinx moth *Manduca sexta* persists throughout postembryonic life. Journal of Neurobiology. 1987;**18**(5):451-465

[87] Sun XJ, Tolbert LP, Hildebrand JG. Ramification pattern and ultrastructural characteristics of the serotonin-immunoreactive neuron in the antennal lobe of the moth *Manduca sexta*: A laser scanning confocal and electron microscopic study. The Journal of Comparative Neurology. 1993;**338**(1):5-16

[88] Sanes JR, Hildebrand JG. Acetylcholine and its metabolic enzymes in developing antennae of the moth,*Manduca sexta*. Developmentaal Biology. 1976;**52**(1):105-120

[89] Homberg U. Immunocytochemical demonstration of transmitter candidates in the central olfactory pathways in the sphinx moth *Manduca sexta*. In: Døving KB, editor. Proc Xth International Symposium on Olfaction and Taste (ISOT X). Oslo, Norway: University of Oslo; 1990. pp. 151-158

[90] Sanes JR, Prescott DJ, Hildebrand JG. Cholinergic neurochemical development of normal and deafferented antennal lobes during metamorphosis of the moth. Brain Research. 1977;**119**(2):389-402

[91] Maxwell GD, Tait JF, Hildebrand JG. Regional synthesis of neurotransmitter candidates in the CNS of the moth *Manduca sexta*. Compound Biochemistry Physiology C. 1978;**61C**(1):109-119

[92] Hildebrand JG, Hall LM, Osmond BC. Distribution of binding sites for 125I-labeled alpha-bungarotoxin in normal and deafferented antennal lobes of *Manduca sexta*. Proceedings of the National Academy of Sciences of the United States of America. 1979;**76**(1):499-503

[93] Waldrop B, Hildebrand JG. Physiology and pharmacology of acetylcholinergic responses of interneurons in the antennal lobes of the moth*Manduca sexta*. Journal of Comparative Physiology. 1989;**164**:433-441

[94] Tumlinson JH, Brennan MM, Doolittle RE, Mitchell ER, Brabham A,

Mazomenos BE, et al. Identification of a pheromone blend attractive to *Manduca sexta* (L.) males in a wind tunnel. Archives of Insect Biochemistry and Physiology. 1989;**10**(4):255-271

[95] Martin JP, Beyerlein A, Dacks AM, Reisenman CE, Riffell JA, Lei H, et al. The neurobiology of insect olfaction: Sensory processing in a comparative context. Progress in Neurobiology. 2011;**95**(3):427-447

[96] Riffell JA, Hildebrand JG Adaptive processing in the insect olfactory system. In: von der Emde G, Warrant E (eds) The Ecology of Animal Senses. Springer; 2016. pp. 3-24

[97] Lei H, Oland LA, Riffell JA, Beyerlein A, Hildebrand JG. Implications from microcircuits of a moth antennal lobe for olfactory information processing [updated version]. In: Shepherd G, Grillner S, editors. Handbook of Brain Microcircuits. Oxford University Press; 2018. pp. 333-344