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FUNCTION AND MORPHOLOGY OF THE ANTENNAL LOBE: New Developments

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■ **Abstract** The antennal lobe of insects has emerged as an excellent model for olfactory processing in the CNS. In the present review we compile data from areas where substantial progress has been made during recent years: structure-function relationships within the glomerular array, integration and blend specificity, time coding and the effects of neuroactive substances and hormones on antennal lobe processing.

INTRODUCTION

The olfactory system plays a very important role for survival and reproduction in the large majority of insects. Sexual partners are located via sex pheromones, food plants are found via kairomones, conspecifics can be gathered using aggregation pheromones, oviposition can be deterred or induced by oviposition pheromones, and nectar-rich flowers are found using synomones. In all of these interactions and many more the olfactory system is indispensable.

Here we endeavor to review progress in studies of the primary olfactory center of the insect central nervous system, the antennal lobe (AL). The morphology and physiology of the insect AL and its neuronal elements has been extensively reviewed in the past (5, 25, 56, 63, 68, 103, 122). Recent years have, however, seen substantial progress in certain areas. The coding of odor blends has now been studied in a number of species and some general conclusions can be reached. The encoding of fluctuating odor concentrations is another area where new results from different organisms provide a more comprehensive view of the neural mechanisms underlying time coding. The functional significance of olfactory glomeruli has been investigated in several species, and also here general patterns have been established. Finally we will discuss the influence of some neuroactive substances and of hormones on AL information processing. In the introduction the stage will be set for the more detailed discussions in the following paragraphs.

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The Antennal Lobe and its Glomeruli

The AL of insects is a sphere-shaped part of the deutocerebrum which receives sensory input from olfactory receptor neurons (ORN) on the antennae and mouth parts (Figure 1*a*) The anatomical and physiological organization of the lobe and its neuronal elements have similar features as primary olfactory centers in other organisms throughout the animal kingdom. The insect AL, as all other primary olfactory centers, consists of so called glomeruli, spheroidal neuropilar structures, housing synaptic contacts between receptor axons and AL interneurons. The arrangement and number of glomeruli within the AL are largely species specific. The number of glomeruli varies from about 32 in the mosquito *A. aegypti* (12) to more than 1000 in locusts and social wasps (41, 61). In most insect species the AL contains 40 to 160 individually identifiable glomeruli arranged in one or two layers around a central fibrous core (8, 12, 23, 121–123, 132). The small glomeruli of locusts and social wasps, which are arranged in a multiglomerular layer around a central fiber core are, however, not individually identifiable (41, 61). Glomeruli are to different extents separated from each other by glial processes (48a, 139).

In a number of well-studied insect species using sex-pheromone communication, a sexual dimorphism in glomerular structure has been observed. The AL of male Lepidoptera, Dictyoptera, and Hymenoptera contains one or several enlarged glomeruli in addition to the sexually isomorphic glomeruli, which are also present in females (for review see 122). The enlarged glomeruli form the macroglomerular complex (MGC) in moths and bees and the macroglomerulus (MG) in cockroaches. The MGC or MG exclusively receive input from sex pheromone–sensitive ORNs (8, 19, 26, 52, 64, 121–123). These findings led to the hypothesis of a functional identity of individual glomeruli, which will be discussed further in this paper, concerning structure-function relationships in ORNs and AL interneurons.

Olfactory Receptor Neuron Anatomy and Physiology

ORN axons originating from cell bodies in the antennae enter the AL through the antennal nerve (Figure 1*a*). In most insects the ORNs arborize in a peripheral layer within one single ipsilateral glomerulus (Figure 1*b*) (for review see 68, 103). Some glomeruli in the honey bee, in some cockroaches, and in the moths *Manduca sexta* and *Mamestra brassicae* do, however, receive ORN terminals penetrating the entire glomeruli (8, 18, 69, 121). In flies ORNs arborize bilaterally and terminate in single corresponding glomeruli in each AL (132). In some Orthoptera species single ORNs arborize in several small glomeruli (Figure 1*c*) (41, 59; Ignell, Anton, and Hansson, personal observation).

ORNs respond very specifically to odors, e.g. to single sex pheromone components (51). Traditionally, ORNs have been characterized as specialists or generalists, where the specialists were examplified by the sex pheromone-specific

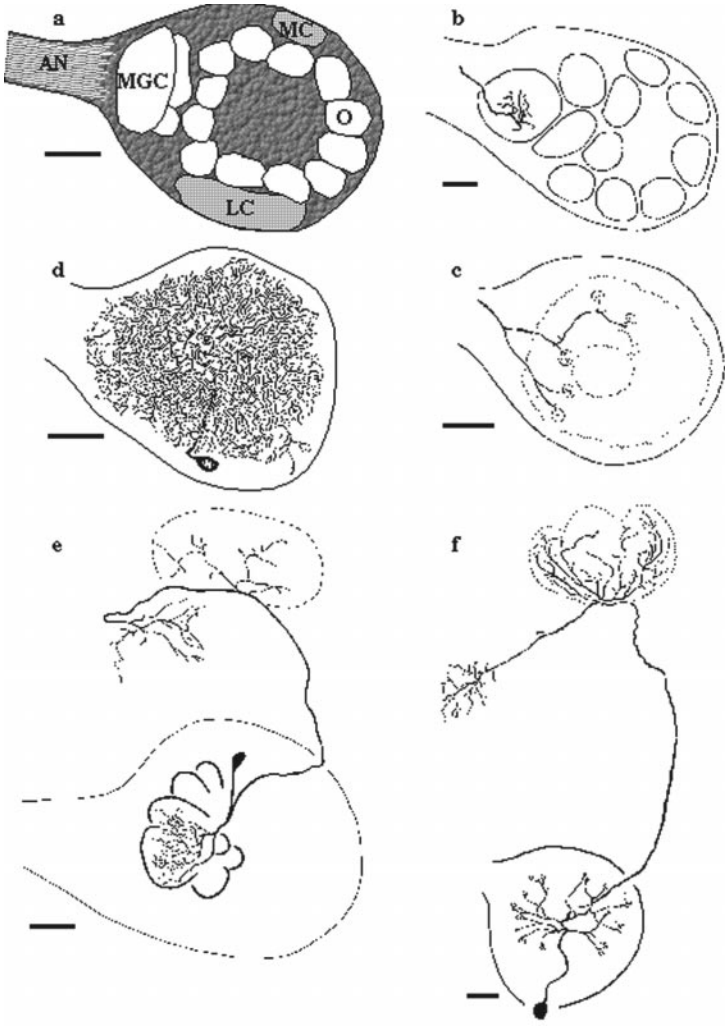

Figure 1 (*a*) Schematic representation of the antennal lobe. The antennal nerve (*AN*) enters from *top, left.* In the antennal lobe the glomerular array is represented by ordinary glomeruli (*O*) and the macroglomerular complex (*MGC*). The MGC is found only in the receiving sex (typically the male) in species using long-distance pheromones. In the periphery of the antennal lobe the two main cell body clusters are situated laterally (*LC*) and medially (*MC*). (*b*) Branching patterns of a pheromone-sensitive olfactory receptor neuron in the antennal lobe of a *Spodoptera littoralis* male. Reconstruction from frontal sections. Scale bar 100 μm. (*c*) Multiglomerular projection of an ORN in *Schistocerca gregaria.* Reconstruction from frontal sections. Scale bar 100 μm. (*d*) Branching pattern of a local interneuron in a *Spodoptera littoralis* female. Reconstruction from frontal sections. Scale bar 100 μm. (*Continued on page 206*.)

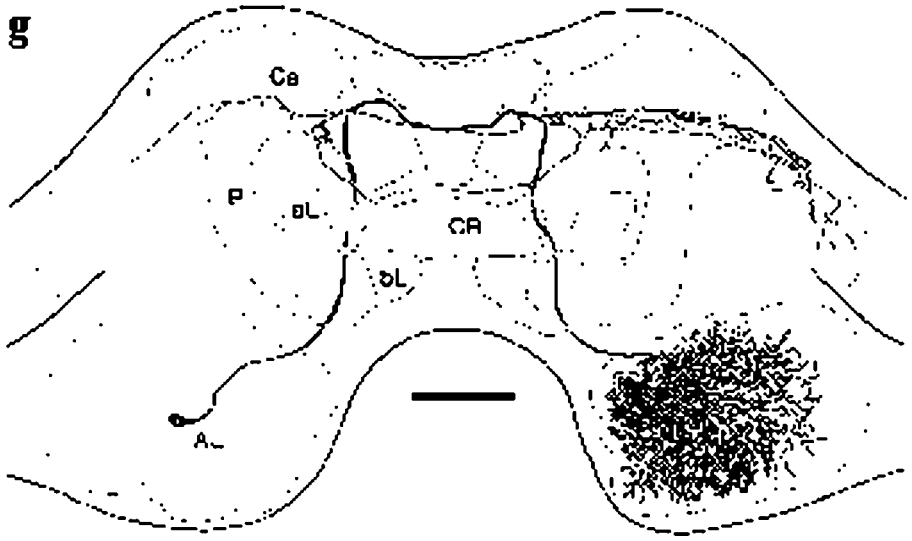

Figure 1 (*e*) Projection neuron arborizing within MGC glomeruli a and b in the antennal lobe of a *Trichoplusia ni* male. The axon runs through the median antenno-cerebral tract, arborizes in the calyces of the ipsilateral mushroom body (dashed outline) and in the inferior lateral protocerebrum. Reconstruction from frontal sections. Scale bar 50 μm. (*f*) Projection neuron with multiglomerular arborizations in *Schistocerca gregaria.* The axon runs through the median antenno-cerebral tract, arborizes in the calyces of the ipsilateral mushroom body (dotted outline) and in the inferior lateral protocerebrum. Reconstruction from frontal sections. Scale bar 100 μm. (*g*) Serotonin immunoreactive centrifugal neuron in the antennal lobe of the sphinx moth, *Manduca sexta.* The neuron arborizes in the superior protocerebrum from where an axon extends into the contralateral antennal lobe, where it ramifies in all glomeruli. Calyces (*Ca*), peduncle (*P*), alpha lobe (*aL*) and beta lobe (*bL*) of the mushroom body, central body (*CB*) (81).

ORNs in moths, and generalists were found among food odor detectors. Today, more examples of extremely specific food- and oviposition-site-detecting ORNs are reported. Most likely, very few generalistic ORNs exist.

Antennal Lobe Interneurons and Their Synaptic Interactions

Three types of AL interneurons have branches within the glomeruli: intrinsic AL neurons [local neurons (LN)], output neurons [projection neurons (PN)], and centrifugal neurons. Most AL neurons have their cell bodies in a number of cell clusters at the periphery of the AL. The location of the cell clusters varies between species (for review see 5). Some cell bodies of PNs and centrifugal neurons are situated in the protocerebrum or in the ventral nerve cord. LNs and PNs both receive direct synaptic input from ORNs. PNs also receive indirect input from

ORNs via LNs (20, 35–37, 99, 100, 136). Output and input synapses have been identified between LNs and PNs and between neurons of the same type. ORNs receive synaptic input from LNs, but not from PNs (20, 35, 37, 100, 136). Synaptic connections of centrifugal neurons have not been studied so far.

Local Neuron Anatomy and Physiology

Three types of LNs have been described in different insect species. Multiglomerular LNs with homogenous arborizations throughout the AL (Figure 1*d*) can be distinguished from multiglomerular LNs with heterogenous arborizations, asymmetrically distributed within the glomeruli of the AL, and from oligoglomerular LNs with branches in only few glomeruli (2, 32, 40, 42, 55, 90, 104, 131, 134).

LNs display different spontaneous activity and different response patterns to odors in different insect species. In the sphinx moth, *M. sexta,* LNs generally seem to have a bursting spontaneous activity, while only some LNs in noctuid moth species show such an activity (2, 3, 32, 55). In bees no bursting activity was found in LNs (42). Intracellular recordings from LNs often show different spike sizes, which might result from several spike initiating zones (32). In *M. sexta* LNs odor stimulation can elicit three different response types. Stimulation of most LNs results in a short-latency excitatory response, while a few neurons show a delayed excitatory or inhibitory response to odor stimulation. Both short-latency and delayed excitatory responses are followed by an inhibitory period (32). In locusts only non-spiking LNs have been described so far. These LNs respond to odors with graded potentials and membrane potential oscillations, oscillations forming a base for synchronization of PNs and protocerebral neurons (91, 96).

A large proportion of LNs in different insect species studied so far show GABA-like immunoreactivity, supporting the physiological findings that output from LNs is inhibitory (13, 32, 34, 68, 72, 93, 125, 129, 145).

Projection Neuron Anatomy and Physiology

PNs usually have their cell bodies in cell clusters in the periphery of the AL. Dendritic arborizations of PNs are either uniglomerular or multiglomerular within the AL, and the axons leave the AL via a number of antenno-cerebral tracts (82), connecting the AL with different areas of the protocerebrum, most prominently the calyces of the mushroom bodies and the lateral protocerebrum (Figures 1*e-f*). The most common type of PN in different insect species has uniglomerular arborizations (multiglomerular in the locust) and the axon projects through the inner antenno-cerebral tract (IACT) to the calyces of the mushroom bodies and to the lateral protocerebrum (for review see 5). PNs leaving the AL through the middle antenno-cerebral tract (MACT) have multiglomerular arborizations within the AL and have been found in moths, flies, and bees (43, 70, 132). Their axons

project to the lateral protocerebrum and to areas adjacent to the pedunculus of the mushroom body. Uni- and multiglomerular PNs leave the AL through the outer antenno-cerebral tract (OACT) in different insect species, including moths, bees, and flies (43, 55, 69, 70, 101, 132). Their axons terminate unilaterally or bilaterally in different areas of the lateral protocerebrum and in some cases also in the calyces of the mushroom bodies. PNs leaving the AL through the dorsal antenno-cerebral tract (DACT) have only been described in the sphinx moth so far (70, 78). These PNs have their cell body in the protocerebrum, have multiglomerular arborizations in the contralateral AL, and project their axon to the lateral horn of the protocerebrum. Very few PNs leaving the AL through the dorso-medial antenno-cerebral tract (DMACT) have been described (78, 132). These neurons have their soma in the subesophageal ganglion and innervate single glomeruli in both ALs. In *M. sexta*, the axon projects to the calyces of the mushroom bodies and to the lateral horn of the protocerebrum.

The response characteristics of PNs have mainly been studied in moths, cockroaches, bees, and locusts. In all species studied, PNs with excitatory responses to certain odors were described, but the exact time pattern of the response varies. In locusts and in the noctuid moths *Helicoverpa zea* and *Heliothis virescens*, inhibitory responses to odor stimulation occurred and the same odors elicited either excitatory or inhibitory responses in different neurons (4, 75, 142). In the sphinx moth, one of the two behaviorally active sex pheromone components elicits an excitatory response in certain PNs, whereas the second component elicits an inhibitory response (25, 26, 29). The spontaneous activity and response of PNs to odors exhibit equally spaced action potentials in *M. sexta*, in contrast to burst-like activity in LNs (32). In noctuid moths, this difference in physiological characteristics has not been consistently found (3; S Anton & BS Hansson, in preparation).

Centrifugal Neuron Anatomy and Physiology

Centrifugal neurons have been studied in a number of insect species including bees, cockroaches, moths and locusts, primarily with immunocytochemical staining methods (Figure 1*g*). The cell body of centrifugal neurons is usually situated outside the AL, in the protocerebrum, in the SOG or in the ventral nerve cord. Exceptionally, serotonin-immunoreactive centrifugal neurons with their cell bodies in the AL cell clusters were found in *M. sexta* and *P. americana* (81, 124). While the dendritic branches of centrifugal neurons can receive input in different areas of the brain or the ventral nerve cord, they exhibit multiglomerular varicose branching within the AL, but can have other output areas in addition (see 5). Both descending neurons from the protocerebrum and ascending neurons from the ventral nerve cord send axonal branches into the AL, but also centrifugal neurons with more restricted arborization areas have been found (21, 49, 65, 66, 118, 132). Neuroanatomical findings suggest a modulatory function of centrifugal neurons, which will be discussed below.

FUNCTIONAL CORRELATES IN THE GLOMERULAR ARRAY

Antennal Lobe Projections of Olfactory Receptor Neurons

Ever since the discovery of glomeruli in different olfactory systems, a general question has been whether these structures have a functional meaning. In insects we have reached far in answering this question. In 1987 Koontz & Schneider (88) could show that the MGC is targeted exclusively by pheromone-detecting receptor neurons in a male moth. More or less simultaneously, Christensen & Hildebrand (26) showed that the same structure was innervated by PNs responding to antennal stimulation with pheromone. A strong case for a functional separation between the MGC and sexually isomorphic glomeruli was thus present.

In the beginning of the 1990s, a method to stain single physiologically defined neurons was developed (58). Using this method, investigators could determine how the axonal arborizations of ORNs tuned to different pheromone components distributed themselves among the glomeruli of the MGC. A number of studies based on this method have now been published and have also been reviewed in detail elsewhere (14, 52, 54, 58, 120, 137). The main result from all of these studies is that a clear odotopic projection pattern of pheromone-specific ORNs is present in more or less all species studied to date. Each glomerulus of the MGC receives input from one type of ORN. Information regarding behavioral antagonists in the pheromone communication system is also received in MGC glomeruli, and ORNs tuned to these compounds subsequently target other specific glomeruli of the MGC.

A very good correspondence between the number of pheromone components and antagonists used by a species and the number of glomeruli included in its MGC has also been shown. We can thus state that there is high probability that olfactory glomeruli are specific projection stations for receptor neurons displaying the same olfactory specificity. These results coincide very well with what was later shown in transgenic mice (109). Olfactory receptor neurons expressing a specific receptor molecule all target the same glomeruli.

Glomerulus Innervation by Antennal Lobe Neurons

What happens at the next neural levels of the antennal lobe? The incoming message is transferred synaptically from ORNs to LNs and to PNs (35–37). LNs often target a majority of the AL glomeruli (104), so from these neurons very little is to be gained in the discussion of specific innervation patterns, except that they can definitely distribute incoming signals all over the AL. PNs, on the other hand, often show uniglomerular dendritic innervation (26, 79) and can thus be of great interest in a comparison with the identity of ORNs innervating the same glomerulus. These innervation patterns have been compared in a number of species, and the results are not as clear as for the ORNs. The first detailed study of PN inner-

vation patterns in the MGC was performed in *M.sexta* (57). A clear pattern was observed, showing a functional projection pattern also in the PNs. PNs responding to the same pheromone component always targeted the same MGC glomerulus. A drawback was, and still is, the lack of information regarding the exact projection patterns of pheromone-specific ORNs.

A number of investigators have now attempted to compare PN dendritic innervation patterns with well-established ORN branching patterns. The results from different studies do not agree unanimously, and totally consistent results should not be expected in biological systems. In *Heliothis virescens*, results from a comparison of twelve physiologically defined and morphologically reconstructed PNs with well-established branching patterns of ORNs show that the PNs always innervate the glomerulus targeted by ORNs expressing the same physiological specificity as the PN (14, 54, 142). Some of the PNs also innervated MGC glomeruli that were not expected from their physiology (142).

In another noctuid moth, *Trichoplusia ni*, a different pattern has been established (4a). This moth uses a very complicated communication system involving seven different types of ORNs. When the innervation patterns of ORNs and PNs were compared, only part of the PNs branched in the glomerulus expected from their physiological characteristics. For PNs responding to the main pheromone component, about two-thirds branched as expected, but for PNs responding to minor components or to a behavioral antagonist none to one-third of them branched in a glomerulus innervated by ORNs of the same specificity. Many neurons also innervated several glomeruli and vice versa.

Optical Imaging of Antennal Lobe Activity

A dream for researchers working on the functional morphology of the AL has been to be able to map olfactory responses over the AL glomeruli in real time. This dream has now come true thanks to the development of optical imaging techniques and their adaptation to the insect system. Optical imaging utilizes changes in, for instance, calcium concentration or voltage and converts these changes to changes in light intensity. By advanced image capture and processing techniques, the minute changes in light emission can be observed and quantified. The pioneers in this research (45, 46, 76) have shown how very distinct activity patterns can be registered in the honey bee AL after stimulation with different odors. A single odor is normally not represented by activity in a single glomerulus, but rather by activity in a number of glomeruli. Obviously a single type of molecule interacts with a number of different types of ORNs resulting in a spatial representation of the odor among the AL glomeruli (76). The response to an odor is not static over time, but can develop as a changing pattern, moving from one pattern to another as time progresses after stimulation. By use of the optical imaging technique it has also been possible to show that the coding of odors is bilaterally symmetric in the two ALs (46).

Functional Correlates in the Glomerular Array: Conclusions

An odor is represented as a spatial map, usually over a number of AL glomeruli. These maps are most likely formed as a consequence of the ability of a single odor molecule type to interact with different types of ORNs expressing different receptor sites in their dendritic membranes. Single glomeruli are clearly the target site for specific ORN types. One glomerulus—one ORN type. As mentioned, this fact now has support from other olfactory systems. What happens after this level? Here we see differences between species. Some species seem to preserve the odotopic patterns established by the ORNs also in the PN innervation patterns; others seem to have less odotopically influenced dendritic innervation patterns among their PNs. Maybe none of these solutions should be any surprise. We know that incoming signals turn glomeruli into ''information packages,'' with each glomerulus holding information regarding a single or a few odors. We also know that this message can be widely distributed over the entire AL by LNs that are often the first postsynaptic element to ORNs. Obviously, through evolution some species have maintained a strictly chemotopically organized system also at the PN level, while others have for some reason let this pattern dissolve into a morphologically less organized system. The neural basis to achieve both these solutions is clearly present.

CODING OF ODOR BLENDS

Rarely do insects rely on single odors as key stimuli releasing a specific behavioral sequence. Whether the odor cues are involved in food, partner or oviposition site search, they usually come as a bouquet composed of a number of chemical constituents. The importance of odor blends has been studied in detail, especially in the sexual communication systems of moths. Many moth species have had their female-produced, long distance pheromones identified in detail (7), and in the great majority the pheromone consists of a number of components, each adding to the attractivity of the blend. The importance of the ratios of components within the blend varies from species to species, where some species have a very narrow response window and others accept a wide range of component ratios.

Also in other behavioral contexts blends of single odors have been shown to play a very important role. In female cotton leafworm moths, *Spodoptera littoralis*, oviposition is deterred by an oviposition deterring pheromone (ODP) released from the feces of conspecific larvae. The ODP consists of six components, and they all must be present to conserve the activity of the pheromone (1). The Colorado potato beetle, *Leptinotarsa decemlineata,* identifies its host by the composition of a number of six-carbon compounds, so-called green leaf volatiles. These compounds are emitted by almost every green plant, but the ratio between the components constitutes a ''fingerprint'' of different plant species. The potato

odor blend *and* component ratio is thus of crucial importance for the food search in *L. decemlineata* (143).

All of these systems, relying on blends of odors, raise a certain demand on the olfactory system. Not only must single odors be identified, but the presence of a combination of odors, sometimes in specific ratios, must be signaled. Such a task can be solved at different levels of the olfactory system, from peripheral receptor neurons to nerves innervating muscles of executing organs such as wings, legs, or ovipositor. At the receptor neuron level, very few studies have shown interactions between single components. Generally, receptor neurons are tuned to single compounds or a range of compounds, but the response of the neuron is not affected by the presence of several different molecules (51). Exceptions to this rule have been presented in the food odor detecting system of some insects (47), but so far never in pheromone detecting ORNs.

The identification of blend configuration thus resides at higher neural levels, from the antennal lobe and onward. To achieve blend specifity, i.e. that the response of a neuron to the blend is different from the summed response of its components, cross-fiber patterns must be present in the nervous system. From many studies we know that labeled lines, carrying information regarding single components, remain present also at very high levels in the CNS (25, 31, 55, 56). The response to a blend can either be stronger than the summed responses to the single components (synergism, Figure 2), or weaker (suppression). In a number of moth species, neurons that show no or a very low response to single pheromone components but that are strongly excited by the complete pheromone blend, have been found. In both the heliothine moths *H. zea* and *H. virescens*, blend-specific PNs have been identified that respond weakly to one or both components of the pheromone. These responses are often short bursts of action potentials. When the blend of the two components is used to stimulate the antenna, however, the same neurons show strongly synergistic responses with long-lasting excitation that outlasts the stimulus presentation period by several seconds (24, 30–31).

Blend-Detection in Polymorphic Systems

The blend specificity described in heliothine moths prompted studies in other species exhibiting different traits in the pheromone communication system. One trait that makes blend specificity especially interesting is when a species displays polymorphism in its pheromone communication system, i.e. different populations utilize different compounds or different proportions of the same compound to ensure sexual communication. Such polymorphism has been described in a number of species. One of the more well studied is the European corn borer, *Ostrinia nubilalis.* In this species the female produces a mixture of the two isomers of 11-tetradecenyl acetate (11–14:OAc). One strain produces and is attracted to the Z and the E isomer in a 97:3 ratio (Z-strain), while the other uses a 1:99 ratio (E-strain) (86). In areas of co-occurrence, the two strains interbreed, producing hybrids emitting and responding to intermediate isomer ratios (87, 149, 150).

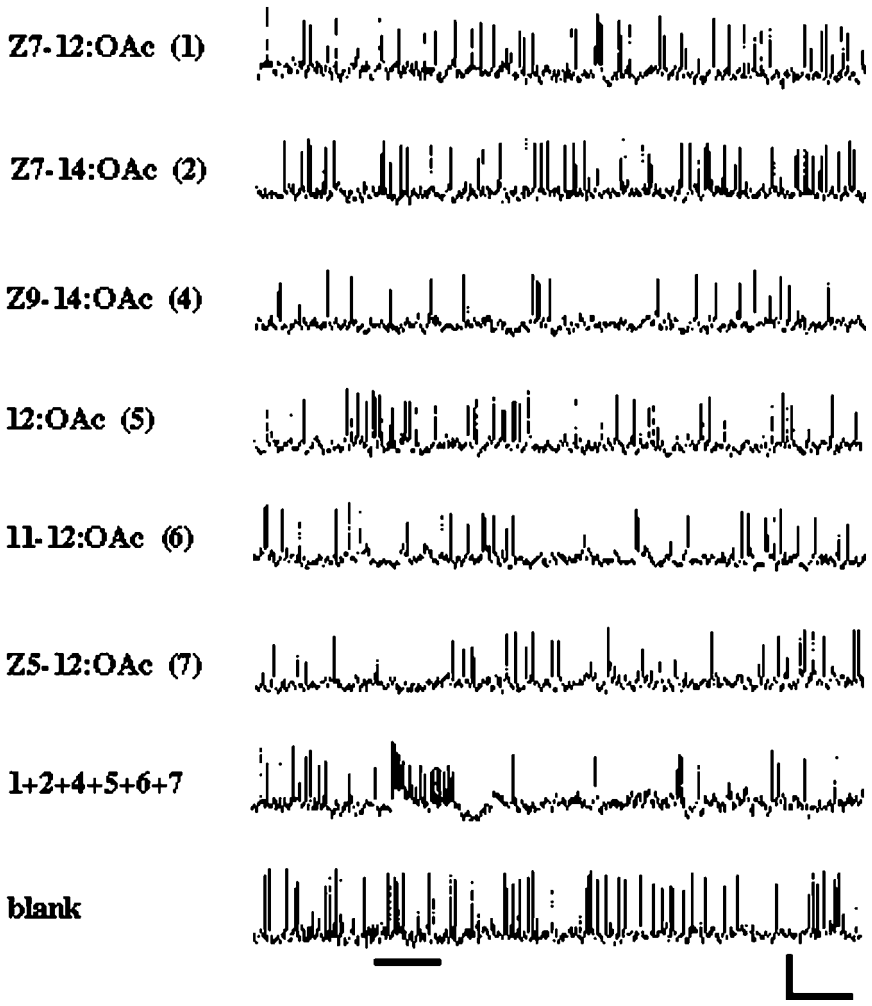

Figure 2 Response characteristics of a blend-specific neuron in *Trichoplusia ni*. The neuron responded to the six-component mixture at 10 ng but not to the single components (1, 2, 4–7). The bar underneath the registration marks the stimulus duration. Vertical scale bar 10 mV, horizontal scale bar 500 ms.

In order to elucidate differences in AL blend specificity between the different strains and the hybrids formed between them, AL neurons were penetrated with intracellular electrodes, and the responses to pure pheromone components, to the parental blends and to some intermediate, hybrid blends were recorded. Synergistic blend specificity was found in 11 out of the 100 investigated neurons. Blend specificity expressed as suppression was found in 40 neurons. Among the blend-

specific neurons were neurons specific to both the parental blends and to the hybrid blends. In the parental strains, neurons responding to ''their own'' blend were more common than those responding to the blend of the other strain. Neurons specific to the hybrid blends were found in only the AL of hybrid males. Among the 49 percent non-blend–specific neurons, a clear correlate was also found, where the male AL of each parental strain contained more neurons tuned to the main component of ''their own'' blend. The arsenal of pheromone-specific AL neurons thus correlated well with the blend produced by females belonging to the same strain (6).

Another polymorphic pheromone communication system has been demonstrated in the turnip moth, *A. segetum.* In this species a large variation both in female pheromone production and male sensory setup was initially shown between different parts of Europe and western Asia (60, 95). However, the largest differences were found when two races, occurring north and south of the Sahara, were compared (140). North of the Sahara the pheromone blend consists of four components in a 1:5:2.5:0.1 ratio. South of the Sahara the blend is radically different, a 1:0.25:0.03:0.1 ratio. The turnip moth is thus an excellent candidate species for testing blend specificity.

When responses of AL neurons were investigated in the two races, neurons displaying blend interactions were found in both (148). In the European population 58% of the neurons showed interactions. Of these, 14% showed synergism, while 44% showed suppression. In the African population the percentages were 9% and 19%, respectively. Again, more than half of the neurons of the European population show blend interactions. This result is most likely the product of a very careful analysis and the use of a large number of blends; in the European population two four-component blends, three three-component blends and six two-component blends were tested, while only four four-component blends were tested in the African population. The more complete the stimulus set, the more interactions will most likely be revealed.

When the responses of the neurons were studied in detail, a large number of interactions were revealed. In 14 of the neurons, the response spectrum allowed a European male to separate the four-component blends of the two populations. Thirteen of these neurons responded with an excitation to the European blend while remaining unaffected after stimulation with the African blend. One neuron displayed the opposite response pattern. In the African population, four blend-specific neurons responded to the African blend, while six were excited exclusively by the European blend (148). These differences are interesting in an ecological and evolutionary perspective. Does the dominance of ''European'' neurons in European-population moths and the presence of both types in equal number in African-population moths imply that the African population stems from the European one, and is now diverging? Could the ''simplification'' of the African blend, as compared to the Swedish, be a product of ecological character release, so that fewer competitors in the pheromone communication channel are present in Africa? These questions remain to be answered.

The results from stimulation with binary blends revealed the full extent of interactions between single pheromone component input to the AL. Response patterns that would not be deduced from the responses to single components were registered in more than half of the neurons after binary blend stimulation. In some neurons only suppressions were present, in others some binary blends resulted in suppression while others resulted in synergistic responses. Still, in others, only synergistic responses were found. This enormous diversity in response characteristics adds another proof of the extensive integrative properties of AL neurons.

Blend Specificity in a Pheromone Communication System Showing Redundancy

In the noctuid moth *Trichoplusia ni* another speciality of the pheromone communication system makes it a good model to study integration of different combinations of pheromone components. The females of this species produce a very complex pheromone blend (94). The complete, behaviorally active sex pheromone of *T. ni* consists of six components (15, 17). Wind-tunnel experiments have shown, however, that several four-component blends elicit full expression of pheromone-mediated behavior in *T. ni* males (94). The pheromone communication system of *T. ni* thus displays what has been termed ''redundancy in the pheromone communication channel.'' Additionally, (*Z*)-7-dodecenol (Z7–12:OH) has been shown to act as a behavioral antagonist (105, 141).

In an investigation of male antennal lobe neurons 112 pheromone-responding neurons were stimulated with single components, with binary blends, with four-component blends and with the full six-component blend. Of the tested neurons 60% exhibited blend interactions. As described previously, two types of blend-specific neurons were present: neurons expressing suppression and neurons expressing synergism. Forty-one neurons (36%) displayed suppression of some kind. Out of these, seven did not respond to the six-component blend, 15 did not respond to four-component blends, and 19 did not respond to binary blends containing single components to which the respective neuron responded. Twenty-seven neurons (24%) displayed synergism. Out of these, 24 did not respond to any single pheromone component or to the behavioral antagonist, whereas three neurons responded to some single components that were not present in the binary blends analysed. Four synergistic neurons responded to binary blends only, three responded to four-component blends only, and 20 neurons responded only to the complete six-component blend (Figure 2). No synergistic neurons were found that responded to the six-component blend and also to four-component blends (S Anton & BS Hansson, personal observation).

Again a very high proportion of the neurons investigated displayed different kinds of blend interactions. However, the redundancy in the pheromone communication system, where several four-component mixtures can substitute for the full blend, was not mirrored in AL blend-specific neurons. Most synergistic blend-specific neurons responded to the full blend and not to the four-component blends.

Neither among synergistic nor among suppressive neurons was a clear neural correlate to the redundancy found.

Concentration Effects in Blend-Specific Neurons

In several of the species investigated and where blend specificity has been demonstrated, the specificity was shown to be highly dose dependent (6, 62). Highly blend-specific neurons could change their characteristics over a single decadic step in stimulus concentration, from responding strictly to the complete pheromone blend to displaying a more generalistic response to some or all of the single components involved. The response threshold in synergistic blend-specific neurons, however, was generally higher than in component-specific neurons, indicating that blend information might be used within a closer range of the emitter. These results point out the importance of using behaviorally relevant stimulus concentrations in olfactory experiments. If not, much information regarding specificity can be lost.

Blend Interactions: Conclusions

Interactions between different components of a behaviorally relevant blend is a common phenomenon in AL neurons. In all three lepidopteran species where detailed analyses have been performed, more than half the neurons investigated displayed some kind of blend interactions. These high proportions demonstrate the importance of the integrative features of the AL. Early speculations that the AL could be a mere relay center for information on its way to higher, integrative centers of the brain are clearly wrong. In the AL, inputs from many different types of ORNs are received and neural comparisons of many of these inputs form the different output signals of the lobe.

TIME CODING

In a natural situation, due to air turbulence, an odor plume is not homogeneous. Instead, it has a filamentous structure. Packages of odor-laden air are intermixed with odor-free air (113). Temporal fluctuations in odor concentration can have a dramatic impact on searching behavior. Many animals are unable to locate a source of odor unless the stimulus presentation is intermittent. Neural circuits in the AL must thus be able to code events in time along with information concerning quality and quantity.

Wind-tunnel observations have demonstrated that the filamentous structure of odor plumes is of crucial importance for a male moth to proceed toward a pheromone source. If a homogeneous pheromone cloud is presented, the male engages in casting, i.e. across-wind flight without upwind progress, similar to when he loses contact with a normal plume (10, 11, 80, 98). For each filament hitting the antenna, the male performs an upwind surge followed by casting. If the frequency

is high enough, in heliothine moths about 5 Hz (10), the flight becomes more or less a straight line towards the pheromone source.

While flying in a pheromone plume, male antennal ORNs receive intermittent pheromone stimulation with changing intervals. A few meters away from a point source, the filaments are about 100 ms long and separated by about 500 ms clean air (114). This intermittency can be translated into a frequency below 2 Hz. Under such discontinuous stimulation, male moth ORNs in several moth species have been shown to be able to follow stimuli mimicking the temporal patterns in a pheromone plume (9, 77, 102).

Antennal-lobe neuron processing of temporally dynamic information supplied by ORNs has been investigated in only a few species. In *Manduca sexta,* PNs have been observed to fire discrete bursts of action potentials following each stimulus pulse, sometimes up to 10 Hz (27). This encoding of time is strongly dependent on interactions between input from two discrete ORN types, each detecting one of the two pheromone-components (28). Typically, input from ORNs detecting the major pheromone component elicits an excitatory response, while input from ORNs specific to a minor component elicits an inhibitory response. When single components were used as stimuli, pulses could in the majority of neurons not be resolved (28), while stimulation with the two-component-blend resulted in a very good time resolution. The response to the blend stimulation had three clear phases: a short, inhibitory postsynaptic potential (IPSP) followed by a depolarization associated with action potentials. The depolarization was subsequently followed by a second, longer inhibiton. A correlation was found between the amplitude of the IPSP, and the maximum rate of pulsing that the neuron could resolve. These results again confirm the notion that many olfactory PNs respond optimally to a specific odor blend rather than to the individual odorants that comprise the blend. The characteristics of the system are also an example of how lateral interactions between neurons residing in different glomeruli can increase molecular differences between odor signals. A type of blend detection is thus at play also in the time-coding system. Integration of two odor pathways synchronises PN activity to the intermittent input.

In a noctuid moth, *A. segetum*, a similar investigation gave a somewhat different picture of how time is encoded in AL neurons. Instead of the triphasic response pattern encountered in *Manduca,* neurons that were able to encode fast transitions in stimulus intensity were characterized by a biphasic pattern (Figure 3). An initial depolarization was followed by a hyperpolarization. An initial IPSP, as found in *Manduca,* was not observed. A second difference was the fact that time resolution was as good for single components as for the full pheromone blend in *A. segetum* (92).

Time Coding: Conclusions

From the two investigations described here it is clear that different mechanisms are in action in the two olfactory systems. The main difference is the reliance on interaction between different pheromone components for time resolution in one

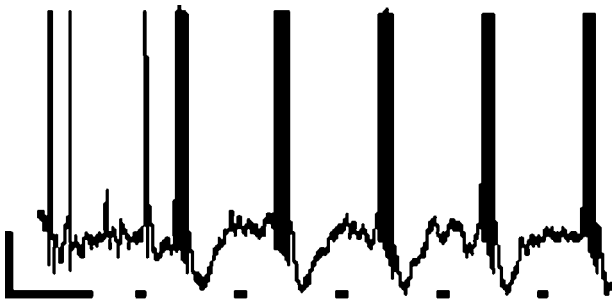

Figure 3 Physiological response of an antennal lobe neuron of the male turnip moth, *Agrotis segetum* when challenged with 2 Hz pulses of the female-produced pheromone blend. The neuron follows the pulses very well. Note the strong inhibitory period following each excitation. Black dashes indicate stimulus pulses (200 ms delay time in the delivery system causes the mismatch between delivery and response). Horizontal scale bar 500 ms. Vertical scale bar 10 mV.

species but not in the other. *M. sexta* uses two main components in its pheromone, while the *A. segetum* female emits a pheromone blend composed of four components. Could the more complex blend of *A. segetum* be the reason why a different strategy is used for time coding? The complexity of a four component blend might preclude the interactions observed in *Manduca*.

NEUROACTIVE SUBSTANCES IN THE ANTENNAL LOBE

A large number of neuroactive substances have been localized within the antennal lobe. They range from neurotransmitters such as acetylcholine (ACh) and γ-amino-butyric acid (GABA) to biogenic amines, neuropeptides and nitric oxide. The majority of the literature on neuroactive substances in the insect brain deals with their immunocytochemical localization within neuropil and identifiable neurons. During the last years, however, the function of some neuroactive substances within the primary olfactory neuropil have been revealed. Here, we summarize the major anatomical and functional findings.

The Neurotransmitters Acetylcholine and GABA

In Lepidoptera, Hymenoptera, and Diptera, acetylcholine or enzymes synthesizing or degrading ACh have been localized in axons of ORNs and in some subpopulations of PNs (for review see 71). The anatomical data suggest that insect ORNs are cholinergic, although functional studies are sparse. In the sphinx moth *M. sexta*, Waldrop & Hildebrand (146) could show in pharmacological experi-

ments that acetylcholine can elicit excitatory or inhibitory responses in AL interneurons.

In most insect species studied so far, γ-amino-butyric acid (GABA) has been localized in LNs (13, 34, 72, 93, 125, 129). Therefore GABA seems to be the main transmitter for LNs, and elicits inhibition in the AL of *M. sexta* (145). Also, some subpopulations of PNs are GABA-ergic in the sphinx moth, the honeybee, and the cockroach (68, 72, 101,125). The effects of GABA, GABA-agonists, and GABA-antagonists on PNs have been studied in some detail. In *P. americana*, GABA injection into the MGC reduced the excitatory responses in PNs to sex-pheromone stimuli, whereas injection of the GABA-antagonist picrotoxin elicited enhanced excitatory responses in the same neurons (20, 74). In *M. sexta*, PNs show responses to odors that are not only dependent on the quality of the stimulus, but also on the temporal pattern (see time coding, above). Pharmacological experiments revealed that GABA plays an important role in shaping the response pattern of PNs to pulsed and to long-lasting odor stimuli through its inhibitory effect. The GABA antagonist bicuculline, which blocks $GABA_A$ receptors/chloride channels in PNs, changed the phase-locked bursting response to pulsed stimuli and the slowly oscillating reponse to long-lasting stimuli into tonic responses, which coded only for the beginning and the end of the whole stimulus (33). In the locust, however, the GABA-antagonist picrotoxin did not change the temporal response pattern in individual PNs, but abolished synchronization of PNs, which is found in non-treated preparations in the form of oscillating field potentials in the calyces of the mushroom bodies (96). Desynchronization of neural activity in the AL of the honey bee through picrotoxin injections impaired the discrimination of similar odorants in learning experiments, which indicates that refined odor discrimination is dependent on GABA-ergic inhibition in the AL (133).

Biogenic Amines

Centrifugal neurons innervating the AL are thought to have feedback and modulatory function. Accordingly, most biogenic amines with potential modulatory function were shown to be present in different types of centrifugal neurons.

Serotonin was localized in centrifugal neurons in moths and cockroaches, where ultrastructural studies revealed output synapses in the AL (124, 135). In *M. sexta*, serotonin was shown to have a modulatory effect on K^+ channels (107, 108). Application of serotonin in the AL led to an increase in excitability connected with an increased input resistance of AL neurons (85).

Dopamine staining revealed processes of centrifugal neurons in bees and crickets (71, 83, 84, 128, 126), whereas no dopaminergic neurons were identified in the AL of flies and locusts (for review see 71). Dopaminergic neurons in the olfactory pathway of honey bees seem to be involved in the retrieval of olfactory memory (106) and in regulating the response threshold to olfactory stimuli in AL neurons (97).

Immunocytochemical staining revealed the presence of octopamine in centrifugal neurons with blebbed branches in the AL in the locust (67), the sphinx moth (67), and the honey bee (89). These neurons have their cell bodies close to the midline of the subesophageal ganglion and send fibers not only into the AL but also in different regions of the protocerebrum. A uniquely identifiable octopaminergic centrifugal neuron (VUMmx1) was shown to mediate the unconditioned sucrose stimulus in olfactory conditioning experiments in the honey bee (49). Depolarization of the VUMmx1 as well as injection of octopamine into the AL or the calyces of the mushroom bodies substituted for the reinforcing stimulus in olfactory conditioning of the proboscis extension reflex (49, 50). Biogenic amines with unknown function have also occasionally been localized in other neuron types in the AL in different insect species (for review see 71).

Neuropeptides

Neuropeptides, such as allatostatins, allatotropin, FMRFamide, and tachykinins were localized in LNs in different insect species with immunocytochemical methods. These peptides are often co-localized with GABA in LNs, but nothing is known about their function in olfactory processing (for review see 71, 117). Neuropeptide-immunoreactivity in ORNs and PNs seems to be rare, whereas neuropeptides with potential modulatory function were shown to be present in different types of centrifugal neurons. Some allatostatin-immunoreactive centrifugal neurons with fibers in the AMMC send processes into the AL in locusts and crickets (126, 144). Centrifugal neurons that descend from the protocerebrum to the ventral cord and send varicose branches into the AL show leucokinin immunoreactivity in locusts and cockroaches (117, 118). Nothing is known, however, about the function of these neuropeptides in the olfactory pathway.

Nitric Oxide

Nitric oxide (NO), a highly reactive free radical gas, has been found to be a mediator for signals in the nervous system throughout the animal kingdom (for review see 112). NO has been found in ORNs in bees, fruit flies, and crickets (110, 114), but seems to be absent in ORNs of locusts (16, 39, 113). The function of NO has been studied in behavioral contexts, such as adaptation processes and learning in bees. NO seems to mediate olfactory information locally (within the ipsilateral AL): Habituation is impaired only ipsilaterally by blocking NO (114). Also, the formation of long-term memory is influenced by blocking NO, whereas short-term memory is not affected (111). In *M. sexta*, the finding that soluble guanylyl cyclase, the target of NO, is expressed especially in PN dendrites, leads to the hypothesis that ORNs might signal directly to PNs via NO within glomeruli, while the main synaptic pathway connects ORNs with PNs via LNs (118).

In different insect species LNs were shown to contain NO (16, 39, 110, 113, 114). In the locust, NO is co-localized with GABA in some LNs (129). However,

NO was not shown to have any effect on odor-evoked synchronization of neuronal assemblies, whereas GABA-antagonists impair synchronization (91, 96).

Hormonal Control in the AL

Recent studies have shown that juvenile hormone (JH) could be involved in plasticity in the AL. In honey bee workers, the change of tasks in the hive is correlated with changes in the JH level and with an increase of the size of an identifiable glomerulus. There is evidence, however, that the plasticity in the size of the glomerulus is not directly dependent on the JH level, but that JH induces foraging activity and this activity leads in turn to an increase in size (130, 147).

Recent electrophysiological studies indicate that JH can affect the sensitivity of AL neurons. In the noctuid moth *A. ipsilon*, newly emerged males are not sexually mature and do not respond behaviorally to the female-produced sex pheromone. The behavioral responsiveness increases with age coinciding with an increasing JH biosynthesis activity (38, 44). Intracellular recordings showed that the sensitivity of AL neurons to the sex pheromone blend increased with the age of the male moths in parallel with the increase in behavioral responsiveness. Males surgically deprived of JH showed a significant decrease in AL neuron sensitivity and high sensitivity could be restored by JH injection. High sensitivity of AL neurons could even be induced in young males by injecting JH. The results indicate that JH plays an important role in synchronizing the development of the reproductive apparatus with the sensitivity of the olfactory system (1a).

In the locust, *Schistocerca gregaria*, JH seems to have the reversed effect on the olfactory system, compared to *A. ipsilon* (Figure 4). The sensitivity of AL neurons to aggregation pheromones in freshly emerged adult individuals is high, whereas AL neurons in four-week-old locusts are often not responding to aggregation pheromones at all. The responses of AL neurons are correlated with behavioral responsiveness to aggregation pheromones, which is high before and during the reproductive time (occuring at an age of two weeks) and clearly decreases thereafter to reach indifferent behavior in four-week-old locusts (Ignell, Couillaud, and Anton, personal observation).

In conclusion, JH seems to coordinate general physiological processes with the regulation of sensory input, but it is still unknown if these effects are directly elicited by JH or if they are mediated by other neuroactive substances such as biogenic amines or neuropeptides.

Neuroactive Substances in the Antennal Lobe: Conclusions

In spite of an increasing rate of identification and localization of potentially neuroactive substances in the insect olfatory pathway, including the AL, the substances with unknown function far outnumber neuroactive substances with a known role in central olfactory processing. Biogenic amines seem to play a similar modulatory role in many different insect species, while the occurrence of neuropeptides varies widely between species and might be responsible for specific

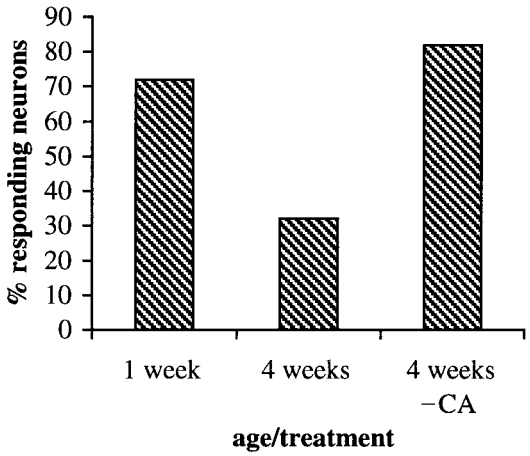

Figure 4 Responsiveness of antennal lobe neurons in male *Schistocerca gregaria* to aggregation pheromone. In young adults, which have a low juvenile hormone level, a large proportion of the neurons respond. In four-week-old adults, having a high juvenile hormone level, only 30% of the neurons respond. In four-week-old adults surgically deprived of juvenile hormone (allatectomised, –CA) the proportion of responding neurons in the antennal lobe was as high as in young adults. Responsiveness of antennal lobe neurons in locusts seems therefore to be controlled by juvenile hormone.

modulatory functions. The different effects of JH in moths and locusts on the sensitivity and responsiveness of AL neurons could be due to the presence of different neuropeptides or other neuroactive substances acting as mediators between the hormone and neuron membrane events. Modulatory substances, such as NO, are also involved in higher brain functions such as learning processes and the formation of long-term memory.

GENERAL CONCLUSIONS

The structural framework underlying AL olfactory processing is the glomerular array. The importance of single glomeruli as target stations for ORNs of identical specificity is becoming an accepted fact, while the innervation patterns of second- and third-level neurons into different glomeruli show a more divergent pattern between insect species and are still under investigation in other organisms. Are different design principles reflecting different odor coding mechanisms? The elucidation of how function is mirrored in the morphology of higher-order neurons of the AL is an important future research direction.

The general function of AL neurons as potent integrators of odor information is clearly very similar between insect species. Input regarding different types of molecules are compared within the AL. As the investigations get more detailed regarding the nature of the stimuli used, the true integrative powers are revealed. In most species investigated, more than half of the neurons show blend interactions. Fast temporal patterns of odor stimulation are followed by AL neurons, a capacity sometimes depending on blend input as well.

As the physiological characteristics of neurons are revealed, the underlying mechanisms of signal transfer and modulation within the AL get increasingly interesting. Our knowledge regarding the function of all the neuroactive substances occurring among AL neurons is still very scarce, and is one of the areas that should be prioritized in future investigations. We already know that quality, quantity, and temporal coding of odors are closely linked, but further studies must also show how the physiological state of insects interferes with these coding mechanisms. By combining the knowledge from morphological, physiological, and pharmacological studies we can reach considerably farther in our understanding of olfactory processing in the antennal lobe.

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CONTENTS