Central Processing of Pulsed Pheromone Signals by Antennal Lobe Neurons in the Male Moth Agrotis segetum

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Lei, H. and B. S. Hansson. Central processing of pulsed pheromone signals by antennal lobe neurons in the male moth Agrotis segetum. J. Neurophysiol. 81: 1113–1122, 1999. Male moths use female-produced pheromones as orientation cues during the mate-finding process. In addition to the needs of evaluating the quality and quantity of the pheromone signal, the male moth also needs to resolve the filamentous structure of the pheromone plume to proceed toward the releasing point successfully. To understand how a discontinuous olfactory signal is processed at the central level, we used intracellular recording methods to characterize the response patterns of antennal lobe (AL) neurons to pulsatile stimulation with the full female-produced pheromone blend and its single components in male turnip moths, Agrotis segetum. Air puffs delivered at frequencies of 1, 3, 5, 7, or 10 Hz were used to carry the stimulus. Two types of AL neurons were characterized according to their capabilities to resolve stimulus pulses. The most common type could resolve at least 1-Hz pulses, thus termed fast neurons; another type could not resolve any pulses, thus termed slow neurons. When fast neurons were excited by stimuli, they always displayed biphasic response patterns, a depolarization phase followed by a hyperpolarization phase. This pattern could be evoked by stimulation with both the single pheromone components and the blend. The pulse-resolving capability of the fast neurons correlated significantly with the size of the hyperpolarization phase. When the amplitude was higher and the fall time of the hyperpolarization faster, the neuron could follow more pulses per second. Moreover, interactions between different pheromone components eliciting different response patterns did not improve the pulse-resolving capability of fast neurons.

INTRODUCTION

In all animals that have developed efficient olfactory systems, odor discrimination is crucial for the organization of feeding, mating, and social behaviors, as well as for learning and memory. Several lines of evidence suggest that the olfactory pathways (from transduction to integration) in vertebrates and invertebrates are comparable (Hildebrand and Shepherd 1997). To understand how olfactory information is processed and subsequently affects behavior, sexual pheromone communication systems in moths and pheromone-mediated flight behavior have been proven to be excellent model systems for investigations (Baker et al. 1989; Christensen and Hildebrand 1987; Hansson 1995; Kennedy 1986). In short, a male moth detects the female-produced pheromone, gets aroused, initiates flight, locks onto the pheromone plume and follows the plume to the female. This series of behavioral events can be affected by many factors. Among them, the spatiotemporal features of the pheromone plume have recently received much attention (Kramer 1986; Mafra-Neto and Cardé 1994; Vickers and Baker 1994; Willis and Arbas 1998).

In a natural situation, due to air turbulence, an odor plume is not homogeneous. Instead, it has a filamentous structure, i.e., packages of pheromone-laden air are intermixed with pheromone-free air (Murlis et al. 1992). Wind tunnel observations have demonstrated that this filamentous structure is of crucial importance for a male moth to proceed toward the pheromone source. If a homogenous 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 (Baker et al. 1985; Baker and Vickers 1997; Kennedy et al. 1980; Mafra-Neto and Cardé 1994). For each filament hitting the antenna, the male performs an upwind surge followed by casting. If the frequency is high enough, in heliothine moths ~5 Hz (Baker and Vickers 1997), the flight becomes more or less a straight line toward the pheromone source.

Flying in a filamentous plume, the male antennae receive intermittent pheromone stimulation with changing intervals. Measured at a distance of 2–15 m downwind of an ionized air plume, the filaments were typically ~100 ms long and separated by ~500 ms clean air (Murlis and Jones 1981). The intermittency of the plume can be translated into a pulse frequency below 2 Hz. Under such discontinuous stimulation, the olfactory receptor neurons (ORN) of male moth antennae have in several moth species been shown to be able to follow stimuli mimicking the temporal patterns in a pheromone plume (Baker et al. 1989; Kaissling 1986; Marion-Poll and Tobin 1992). The axons of the ORNs project into the antennal lobe (AL), which is the primary olfactory center of the moth brain. There they arborize in spherical neuropil, glomeruli. Male pheromone-specific ORNs project to a specialized complex of glomeruli situated at the entrance of the antennal nerve into the AL, the macroglomerular complex (MGC) (Hansson et al. 1992, 1994). From the ORNs, information is synaptically transferred to local interneurons (LN) and to the output elements of the AL, the projection neurons (PN) (Distler and Boeckh 1997a,b).

Only a few studies have been carried out investigating how AL neurons further process temporally dynamic information supplied by ORNs. In Manduca sexta, PNs have been observed to fire discrete bursts of action potentials following each stimulus pulse, sometimes up to 10 Hz (Christensen and Hildebrand 1988). This encoding of time has been shown to be strongly dependent on interactions between input from two discrete ORN types, each detecting one of the two pheromone components (Christensen and Hildebrand 1997). ORNs detect-
ing the major pheromone component elicits an excitatory response, whereas ORNs specific to a secondary component elicits an inhibitory response in many of the pulse following PNs investigated. When single components were used as stimuli, pulses could most often not be resolved (Christensen and Hildebrand 1997), whereas stimulation with the two-component blend resulted in a very good time resolution. The combined response pattern to the blend consisted of three clear phases: a brief, initial inhibitory postsynaptic potential (IPSP) followed by a depolarization associated with action potentials. The depolarization was subsequently followed by a second inhibitory phase. These results showed that in *M. sexta*, the pheromone blend enhanced the capability of AL neurons to encode a pulsatile signal. The size of the IPSP was suggested as the key to enable a PN to resolve stimulus pulses and was related to the inhibitory effect of the secondary component in the pheromone blend (Christensen and Hildebrand 1997). However, more comparative studies are needed to test whether this pattern is a general principle. Moreover, the pheromone blend of *M. sexta* that was used in these studies is only composed of two components, whereas in many other moth species, the blend usually consists of more than two components. For instance, *A. segetum* males are behaviorally attracted by a four-component-blend composed of (Z)-5-decenyl acetate (Z5–10:OAc), (Z)-5-dodecenyl acetate (Z5–12:OAc), (Z)-7-dodecenyl acetate (Z7–12:OAc), and (Z)-9-tetradecenyl acetate (Z9–14:OAc) in a 1:0.1:5:2.5 ratio (Wu et al. 1995). Such a multicomponent blend would be expected to evoke more complicated response patterns than a two-component blend. Nothing is known so far about whether and how the interaction of different pheromone components affects pulse resolution at the AL level in moth species that use multicomponent blends as orientation cues.

In the present study, we approached the question how AL neurons in *A. segetum* males respond to pulsed pheromone stimulation, using an intracellular recording method. AL neurons were classified based on their pulse-resolving capabilities.

**METHODS**

**Insect preparation**

A laboratory culture of *A. segetum* was maintained at the Department of Ecology, Lund University. The pupae were sexed; males and females were kept in separate chambers at a temperature of 23°C and a light period of 17 h. Two- to three-day-old male adults were used for electrophysiological experiments. For intracellular recordings, a male moth was restrained in a 1-ml plastic pipette with the tip cut off to allow the moth head to protrude. The head was immobilized with dental wax (KERR). Scales and cuticle together with proboscis, trachea, and muscles were carefully removed to expose the brain. The AL to be recorded from was manually desheathed with fine forceps to facilitate microelectrode penetration. The exposed brain was perfused with a running saline solution (pH 6.9) (Christensen and Hildebrand 1987) to prevent desiccation.

**Stimulation**

A constant charcoal-filtered and humidified airflow was blown at a speed of 0.5 m/s over the antenna to keep fluctuations in mechanical stimulus to a minimum. The airflow was delivered through a glass tube (8 mm ID) that ended 10 mm before the antenna. Ten nanograms of single pheromone components (Z5–10:OAc, Z5–12:OAc, Z7–12:OAc, or Z9–14:OAc) or the full pheromone blend of these four acetates in a 1:0.1:5:2.5 ratio, dissolved in distilled hexane, were applied on a piece of filter paper (5 × 15 mm) that was placed inside a Pasteur pipette. Distilled hexane on a filter paper was used as blank. The pipette was inserted into the glass tube carrying the constant airflow 150 mm from the antenna. A single puff or a series of puffs of the pipette atmosphere was injected into the airflow by an odor stimulus controller (SYNTECH, Hilversum, The Netherlands) at a flow rate of 5 ml/s. When a neuron was contacted, its specificity was established using 500- or 100-ms single stimulations of each pheromone component and the full blend. The neuron was then challenged with progressively higher frequencies of pulses of the physiologically active compounds at frequencies of 1, 3, 5, 7, and 10 Hz. At frequencies of 1, 3, 5, or 7 Hz, the pulse duration was 100 ms; for the 10-Hz treatment, the duration was 50 ms. Contact quality did not allow all frequencies to be tested in all neurons. In total, 18 neurons were tested with the full spectrum of frequencies and with all effective substances. After the pulse treatment, if the contact was still good, single stimulations of 20, 50, 100, and 200 ms were applied to the neuron to establish the relationship between pulse length and response duration.

**Electrophysiological recording and staining**

Glass microelectrodes were pulled on a Sutter P-87 microelectrode puller. Recordings and stainings were performed according to standard intracellular methods (Christensen and Hildebrand 1987). Neuronal activity was visualized on an oscilloscope (Gould) and recorded on videotape using a modified VCR (Vetter).

**Data analysis**

In response to a stimulus train, a typical response was discrete bursts of action potentials (spikes) intermitted by inhibitory periods. Each stimulus pulse evoked a spike burst (depolarization phase) with 200- to 400-ms delay due to the delivering time of odorants and neuronal processing time and a subsequent inhibitory period (hyperpolarization phase) (see RESULTS). For these neurons, various response parameters were measured: the number of spikes within each discrete burst, the duration of each spike burst, and the amplitude and fall time of hyperpolarization phases (Fig. 2). The hyperpolarizations were measured as the deflection from a mean value of the baseline potential during 300 ms before stimulation. The same parameters were also established after single-pulse stimulations. The results from the single-stimulation experiments were used to correlate the stimulus duration and the duration of the evoked burst (Fig. 9). The results from the pulsed-stimulation experiments were used to correlate the temporal characteristics of the burst train to those of the stimulus (Fig. 7). To establish the influence of the hyperpolarization phase on the pulse-following capability of individual neurons, the first five hyperpolarization phases in the 1-Hz stimulation were measured. The average values of hyperpolarization potentials and potential fall time were calculated and correlated with the pulse-resolving capability of the neuron (Fig. 5). To compare burst duration evoked by pulsed stimulation with different frequencies, the first burst duration evoked by the 1-Hz stimulation was set as 100%, and the successive burst durations were standardized to the first. The first burst durations from other frequency tests were standardized to the one from the 1-Hz experiment and then served as standards for the remaining burst durations (Fig. 7).

Instantaneous spiking frequencies were measured to compare the response patterns of some neurons (Figs. 6 and 8). The measurement was conducted, and the results were plotted using AUTOSPIKE (SYNTECH) program.

The nonparametric Spearman R test was used to test the correlation between pulse-resolving capability and the amplitude of the hyperpolarization phase or the duration of the hyperpolarization fall time (Fig. 5). Only neurons where all frequencies were tested were used in this
analysis. The procedures of this analysis were carried out using STATVIEW software.

RESULTS

In total, the responses of 48 AL neurons to pulsed stimulation were characterized. The neurons were grouped according to their response patterns: 41 could resolve stimulus pulses of at least 1 Hz, thus called fast neurons; 7 neurons could not resolve pulses of any frequency, thus called slow neurons. Among the fast neurons, 33 showed mixed responses and 3 showed exclusively inhibitory responses. These two subgroups were termed fast mixed neurons and fast inhibitory neurons, respectively. Additionally, five neurons responded in different manners to different components, excitatory to some, inhibitory to some. Within the slow neurons, five showed excitatory and two showed inhibitory responses.

Neurons displaying fast mixed responses

This group constitutes 80% of the sampled neurons. In our initial investigation, morphological characterizations were not performed on the neurons studied. However, judged from the duration of action potentials, firing patterns and penetration sites of the electrode into the AL, it is highly likely that all neurons characterized by a fast mixed response were PNs. On contact, LNs often fire clusters of action potentials, whereas PNs display a more random firing pattern. The action-potential duration is also shorter in PNs than in LNs (Hansson et al. 1994). To further strengthen this supposition, a second series of experiments was performed where five neurons displaying the typical fast mixed response pattern were morphologically characterized (Fig. 1). All of these neurons were PNs.

Neurons belonging to this group always showed a biphasic (+/−) response pattern to a stimulus pulse, whether single or within a pulse train (Fig. 2). An initial depolarization phase was accompanied by action potentials (burst of spikes). This phase was always followed by a hyperpolarization phase. In the pulsing experiments, this response pattern resulted in a series of discrete bursts that followed the individual stimulus pulses (Fig. 2, C, D, and F, and Fig. 3). An initial hyperpolarization was never observed, not even after injection of 0.5 nA of depolarizing current. For each response, the burst duration (BD) was measured as well as two parameters to quantify the hyperpolarization phase: the amplitude of the hyperpolarization potential (HP) and the hyperpolarization fall time (HFT), i.e., the time needed to reach the lowest membrane potential from the end of the depolarization phase (Fig. 2B). These two parameters determined the shape and the size of the hyperpolarization phases, as shown in Fig. 2 (compare A with B). When the biphasic pattern was present, stimulus pulses could be resolved. This biphasic pattern was in the large majority of neurons identical when evoked by stimulation with single pheromone components or with the blend (Fig. 3). Neither the burst duration nor the size of the hyperpolarization phase (HFT and HP) were changed due to different stimuli (Fig. 3). In general, neurons that resolved single component pulses could also resolve blend pulses at the same frequency and vice versa. Consequently, stimulus pulses of various frequencies, either carrying single pheromone components or carrying the blend, were resolved by about the same percentage of neurons (Fig. 4). Among the neurons that responded to the single pheromone components or the blend, 100% could resolve 1- and 3-Hz pulses, whereas 30–60% of the neurons resolved 5-Hz pulses (Fig. 4). Very few neurons resolved 7- or 10-Hz pulses. When a given frequency exceeded the pulse-following capability of a neuron, the neuron usually displayed only an initial excitation.
in response to the first stimulus pulse, whereas no response was elicited by the remaining pulses (Fig. 2E). When the frequency was faster, the nonactive period was more prolonged.

Different pulse-resolving capabilities were observed among the fast mixed neurons. Further analysis of the biphasic response patterns revealed that the pulse-following capability correlated positively with the amplitude of the hyperpolarization potential (Spearman $R = 0.656$, $P = 0.0068$; Fig. 5A) and negatively with the hyperpolarization fall time (Spearman $R = -0.691$, $P = 0.0044$; Fig. 5B).

The fast mixed neurons displayed different specificities. When a neuron responded to one or some of the pheromone components, it responded to the blend as well. However, two neurons were blend specific; they did not respond to any single component but well to the four-component blend. These neurons resolved 1- and 3-Hz pulses. Five neurons displayed differential responses, i.e., they were inhibited by some components but excited by others (Fig. 6). Compared with the single components, the blend elicited only a weak excitatory response (Fig. 6F). These neurons resolved pulses up to 3 Hz when supplied with stimulus pulses of single, excitatory components (Fig. 6, C–E). However, they did not follow the blend.
pulses at all (Fig. 6F), even at a frequency of 1 Hz, nor did they follow single component pulses eliciting inhibitory responses (Fig. 6B).

Stimulation with different frequencies resulted in different burst durations (Fig. 7). In general, the burst duration decreased after the first burst, and the higher the burst frequency, the stronger was the decrease in duration. An initial reduction in burst duration was observed in all frequency tests except for the 1-Hz test. In the 3-Hz test, there was ~20% reduction in duration between the first and the second burst, whereas the rest of the bursts remained at the second burst level. For the 5- and 7-Hz stimulation, the reduction went on until the fourth burst that was only ~40% of the first burst's duration.

Burst durations measured from fast mixed neurons varied considerably when stimulating the antennae with the same duration of stimulation (Fig. 9). For stimulation durations of 50, 100, and 200 ms, the mean values of evoked burst durations were longer than the stimulations, whereas for 500 ms duration, evoked burst durations were shorter than 500 ms in average.

**Neurons displaying slow excitatory responses**

This group of neurons constitutes ~10% of the entire sample. Among these neurons a monophasic response pattern was always observed (Fig. 8). Only the excitatory phase was present. The excitation was expressed as two different types, short-lasting excitation (Fig. 8A) and long-lasting excitation (LLE; Fig. 8B). Stimulated by a single pulse of 100 ms, the first neuron type produced spike bursts of ~50 ms, whereas for the second type, the excitation lasted at least 5 s. When the LLE neuron shown in Fig. 8B was challenged with 1-Hz pulses, it displayed an LLE with an oscillatory component. The oscillations were, however, not phase locked to the stimulations. Thus none of the two neuron types displaying a slow excitatory response resolved stimulus pulses at 1 Hz.

**Neurons displaying fast or slow inhibitory responses**

This neuron group constitutes 10% of the whole sample. In response to single stimulation, inhibitory fast neurons responded with a hyperpolarization period that was immediately followed by a restoration of spontaneous activity. The hyperpolarization periods lasted approximately as long as the stimulation. In response to repeated stimuli, this neuron type generated response patterns that appeared as
spontaneous activity intermitted by a series of hyperpolarization periods following stimulus pulses up to 3 Hz. Only one inhibitory slow neuron, not being able to follow 1-Hz pulses, was found.

DISCUSSION

Most AL neurons of the male *A. segetum* are capable of resolving behaviorally relevant pulse frequencies of sex pheromone odors. The neurons do, however, present clearly different capabilities to resolve pulsed signals, ranging from below 1- to 10-Hz frequencies. In *A. segetum*, the key to odor pulse resolution resides in the biphasic response pattern of fast neurons, but not in interactions between inputs regarding different pheromone components.

**Biphasic response pattern**

The great majority of neurons investigated displayed the fast mixed response type. Without exception, these neurons were characterized by a biphasic (+/−) response pattern. This pattern differs from that reported for *M. sexta* where a triphasic −/+/− pattern, i.e., an IPSP followed by an excitation then followed by a second inhibition, was observed (Christensen and Hildebrand 1997). In *A. segetum*, no IPSP was present. No hyperpolarization could be observed, even when +0.5 nA of depolarizing current was injected into investigated neurons. During such current injection, the biphasic patterns were still clearly present, and the neurons resolved odor pulses. We propose that the biphasic response-pattern functions to punctuate the depolarized state of a neuron to make it ready to receive the next stimulus pulse.

**FIG. 5.** Correlation between the size of the hyperpolarization phase and the degree of pulse resolution. The pulse-following capability was positively related to the amplitude of the hyperpolarization potential (HP) (A) but negatively to the hyperpolarization fall time (HFT) (B). Eighteen neurons that were tested with the full range of frequencies are contributing to this graph.
Important factors determining the pulse-following capability of a fast neuron were the characteristics of the hyperpolarization phase. When the amplitude was larger and the fall time of the hyperpolarization phase shorter, the responses were more abruptly punctuated, and thus the neuron could resolve the higher frequency of pulses (Fig. 5).

If we return to the data of Christensen and Hildebrand (1997), the functional characteristics of the response of pulse-following neurons can be directly compared, despite the presence of the initial IPSP in *M. sexta*. If both the initial IPSP and the second hyperpolarization in *M. sexta* were products of the same synaptic input, the amplitude of the initial IPSP could be seen as a measurement both of itself and of the second inhibitory phase, and the second IPSP could potentially have the most important functional significance. Thus the two systems could both work according to the principle of the +/− pattern: a strong response that is promptly punctuated by a pronounced stop signal. If the two hyperpolarizations are of different origin (synaptic vs. intrinsic), the mechanisms of encoding of time in the two systems are indeed different because the initial IPSP is clearly absent in *A. segetum*, and further experiments will have to be performed to clarify the exact functions.

Concerning the +/− response patterns, the question arises what neural mechanisms underlie the two phases of the +/− response observed in neurons showing fast mixed responses. The biphasic response pattern could result from different synaptic inputs. A recent study on cockroaches, *Periplaneta americana*, showed that PNs were postsynaptic to ORNs and to LNss (Distler and Boeckh 1997a,b). The ORN-PN and ORN-LN-PN circuits could be the mechanisms underlying the biphasic response patterns observed in the present study. In response to a stimulus, the ORNs depolarize the PNs via excitatory synapses; meanwhile, the ORNs also excite GABAergic LNss that provide inhibitory input to the PNs (Boeckh et al. 1990; Waldrop et al. 1987). The inhibitory input terminates the depolarization of the PNs and thus allows a sharp punctuation of each stimulus pulse. If this theory were true, one would expect that the duration of the depolarization phase should be more related to when the hyperpolarization phase starts than to the duration of the stimulation. This speculation is supported by our data. When giving stimulations of fixed duration, burst durations varied considerably, especially for the 500-ms stimulations (Fig. 9). One explanation of these variations could be that neural circuits with different time
constants, i.e., with different timing of excitation and inhibition, shape the response pattern of the fast mixed neurons. Alternatively, one can speculate that the biphasic response pattern is an intrinsic property of these neurons. To clarify this, further experimental evidence from pharmacological studies is needed.

Data from neurons displaying a slow response provide further evidence to support the importance of biphasic response patterns for the encoding of time, because these neurons displayed monophasic responses and did not follow any stimulus pulses (Fig. 8). The LLE pattern (Fig. 8B) recorded at the AL level might contribute to the input of some neurons in the lateral accessory lobes in the protocerebrum, where the same response pattern has been observed (Kanzaki and Shibuyo 1992). During the mate-finding process, these neurons may function to signal the mere presence of pheromone and through the LLE serve as a short-term memory of odor impressions.

No blend interactions in the encoding of time

The biphasic response pattern in *A. segetum* was observed both when stimulating neurons with active single pheromone components and with the four-component blend (Fig. 3). The amplitude and duration of the hyperpolarization phase did not differ between the component-evoked responses and the blend-evoked responses (Fig. 3), and the proportion of neurons reaching a certain level of pulse resolution was very similar among the single components and the blend (Fig. 4). These results show that AL neurons in the male *A. segetum* do not depend on blend interactions to resolve odor fluctuations over time. This pattern differs from what has been reported in *M. sexta*, where a large majority of the PNs require input from two distinct ORN populations, each specific to one of the two pheromone components tested, to resolve a pulsed stimulus (Christensen and Hildebrand 1997). In both *M. sexta* and *A. segetum*, it is clear that a mixed response pattern is necessary for most AL neurons to be able to follow a fluctuating stimulus, but blend interactions seem to play a different role in the two species.

Females of *A. segetum* produce four behaviorally attractive pheromone components (Wu et al. 1995), which were all included in the present study. The *M. sexta* female produces two pheromone components of major importance (Tumlinson et al. 1989). This difference in chemical architecture might be one of the reasons underlying the different strategies for temporal resolution observed in AL neurons in these two species. A more complex pheromone system might preclude the two-component interactions observed in *M. sexta*, and instead favor a system where temporally fluctuating input regarding single components is potent to enable pulse following in AL neurons. Several minor pheromone components have been identified in *M. sexta* (Tumlinson et al. 1989), but their influence on the encoding of time by antennal lobe neurons has not been investigated.

The equivalent capabilities of resolving component pulses and blend pulses in neurons characterized by a fast mixed response in the male *A. segetum* AL could indicate that single pheromone components are equally important as the blend in evoking the male search behavior. However, a large number of behavioral observations have shown the importance of the full blend during the pheromone-source-finding process (e.g., Linn et al. 1988; Palaniswamy et al. 1983; Willis and Baker 1988; Witzgall and Arn 1990). A likely explanation to this paradox is that the neural encoding of a pheromone plume consists of many aspects. Most of the neurons function to encode the plume structure and probably the pheromone concentration as well, whereas some of them, such as the blend-specific neurons, are responsible for coding the blend composition. In the blend-specific neurons, the contrast between the optimal and nonoptimal pheromone signal is emphasized (Hartlieb et al. 1997; Wu et al. 1996). Thus, only if information from all aspects (structure, composition, and concentration) is optimal, a full behavioral performance can be induced. Because encoding the plume structure is crucial, male moths use a large number of neurons to implement this task. The ratio of the neurons displaying fast versus slow responses (85:15%) may indi-
cate the importance of encoding plume structure. Moreover, a large difference between the number of AL neurons resolving 1- or 3-Hz pulses and of those resolving 5-Hz or higher frequency pulses is clearly present (Fig. 4). This difference can be viewed in the context of naturally occurring odor plumes. Very seldom will a moth encounter odor
pulses of frequencies \(>3\) Hz (Murlis et al. 1992), so the threshold observed at 3 Hz most likely reflects the performance level required from the male moth olfactory system.

**Conclusions**

In conclusion, the AL neurons of male A. *segetum* can rapidly encode fluctuating odor concentrations, as encountered in a pheromone plume. The processing of pulsed odor stimuli by AL interneurons produces various results. Some of them exactly reflect the temporal features of the signal, whereas others only reflect the presence of the signal; some encode the signal with excitatory responses, whereas others with inhibitory responses; some do not distinguish single component stimulation from a full blend stimulation, whereas others do. These different but parallel neural pathways indicate that a large amount of data regarding the complexity of a pheromone plume is formed at the AL level. It is clear that different moth species use different strategies to encode temporal patterns in odor occurrence. Although the presence of the blend is very important for temporal resolution in odor occurrence. Although the presence of the blend is very important for temporal resolution in *M. sexta*, it is not in A. *segetum*. Although an initial IPSP might be important for time coding in *M. sexta*, it is not present at all in A. *segetum*. These differences are intriguing and prompt further investigations of the encoding of time at different neural levels of the moth olfactory system.

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