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Olfactory signal coding in an odor background

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ABSTRACT

Insects communicating with pheromones are confronted with an olfactory environment featuring a diversity of volatile organic compounds from plant origin. These volatiles constitute a rich and fluctuant background from which the information carried by the pheromone signal must be extracted. Thus, the pheromone receptor neurons must encode into spike trains the quality, intensity and temporal characteristics of the signal that are determinant to the recognition and localization of a conspecific female. We recorded and analyzed the responses of the pheromone olfactory receptor neurons of male moths to sex pheromone in different odor background conditions. We show that in spite of the narrow chemical tuning of the pheromone receptor neurons, the sensory input can be altered by odorant background.

1. Introduction

Olfaction is an essential sensory modality for insects to find a mate, a food source or an oviposition site. It is generally considered that most insects respond in a deterministic way to specific chemical blends released by their host or conspecifics. The parameters of the odor signals that will allow discrimination of the signal and orientation toward its source are the chemical components of the blend and their relative proportions in the blend (quality), their concentration in the air (intensity), and their distribution in time (dynamics). Olfactory signals are detected by olfactory receptor neurons (ORNs) housed in sensilla mainly situated on the insect antennae. Quality, intensity and dynamics of odor signals contain decisive information for insects flying toward odor sources (Cardé and Willis, 2008; Vickers, 2000, 2006). The olfactory coding process starts in the ORNs whose chemical tuning insures specific detection of odorants and coding of intensity and temporality of odor signals into spike firing patterns. However, in natural environments insects are confronted with a rich olfactory world from which their olfactory system must extract the relevant information. Terrestrial plants release in the atmosphere a great variety of volatile organic compounds and a total of 1700 compounds have been identified from floral scents (Knudsen et al., 2006). The mixing ratio of volatile plant compounds in air is typically in the range of several ppb (Kesselmeier et al., 2000; Wiedenmyer et al., 2011). Presence of such large amounts of many volatile organic compounds in the atmosphere may alter the detection of a specific signal often released in much lower concentrations. Insects communicating with pheromones for instance possess specialized olfactory receptor neurons narrowly tuned to the pheromone components (Phe-ORNs). Each pheromone component activates a specific type of Phe-ORN and the recognition of the pheromone blend is achieved by specific coding. However, the chemical specificity of Phe-ORNs can be challenged by a diversity of volatile compounds released by plants (VPCs, Deisig et al., 2014). Different modes of interactions between pheromone components and VPCs have been reported. Ochieng et al. (2002) described synergy between linalool or (Z)-3-hexenol and Z11-hexadecenal in the noctuid moth *Heliothis zea* and hypothesized that the co-perception of pheromone and plant volatile could facilitate the finding of a female. In turn, adding a VPC to the pheromone results more generally in a suppressive effect (Den Otter et al., 1978; Van der Pers et al., 1980) suggesting that a background of volatile organic compounds constitutes an odorant noise that might decrease sensitivity.

The effects of background on extraction of a signal and its consequences on behavior have been largely investigated in the sensory modalities involving physical stimuli like vision (Chen et al., 2014; Sasaki et al., 2006, 2008) and audition (Brumm and Slabbekoorn, 2005; Chan et al., 2010; Schmidt and Römer, 2011; Siegert et al., 2013). In turn, the consequences of an odor background, that can

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be assimilated to a "chemical noise", on perception of the olfactory signals have been far less studied. The effects of VPCs on responses of individual neurons to pheromone have been analyzed (Party et al., 2009; Rouyar et al., 2011), but the consequences on reliability of sensory input at the neuron population level have been poorly documented. In Drosophila larvae, a combinatorial code of odor components involves not only precise lock and key coding of specific odors but also patterns of a stochastic and fuzzy activity in neuron ensembles (Hoare et al., 2008). In the present paper, we aimed to evaluate how much environmental odorants affect the intensity, quality, temporality and reproducibility of the response to a specific signal odorant in ensembles of Phe-ORNs. Many volatile plant compounds that constitute the odorscape of insects contain important contextual information and serve as cues for host plant detection; however, we will consider these environmental odorants as an external background, relatively to the pheromone signal.

We thus recorded, by extracellular single sensillum methods, the firing activity of the Phe-ORNs in the presence of a background of a VPC in two noctuid moths, *Spodoptera littoralis* and *Agrotis ipsilon*. We expected that, firstly, the interactions between background and signal would affect the way intensity, quality and temporality dimensions of the pheromone signal are coded within a population of Phe-ORNs. Secondly, we expected that the interactions between background and signal would increase the variability: all ORNs are not exposed the same way, there is some uncertainty in the timing of the signal/background arrival on ORNs, all ORNs might not be as sensitive to the background. . . To confirm our working hypotheses, the intensity and dynamics of the firing responses of Phe-ORNs to pulses of pheromone in a VPC background were analyzed.

2. Material and methods

2.1. Insects

Larvae of *A. ipsilon* and *S. littoralis* were reared in the laboratory on artificial diets at 23 °C and 60% relative humidity until pupation. Sexes were separated at the pupal stage, and females and males were kept in separate rooms under a reversed 16 h:8 h light:dark photoperiod under similar temperature and humidity conditions. Newly emerged male adults were collected every day and provided *ad libitum* with a 20% sucrose solution. The day of emergence was considered day zero of adult life. Two to five day old sexually mature virgin males were used for electrophysiological experiments, and all electrophysiological experiments were performed during the scotophase.

2.2. Chemicals

2.2.1. Sex pheromones

We used a sex pheromone blend of *A. ipsilon* based on the three previously identified components (Gemeno and Haynes, 1998; Picimbon et al., 1997): (Z)-7-dodecen-1-yl acetate (Z7-12:OAc), (Z)-9-tetradecen-1-yl acetate (Z9-14:OAc) and (Z)-11-hexadecen-1-yl acetate (Z11-16:OAc), mixed at a ratio of 4:1:4 (Causse et al., 1988). The three compounds were purchased from Sigma Aldrich (Saint-Quentin Fallavier, France) and diluted in hexane (>98% purity, CAS 110-54-3, Carlo-Erba, Val-de-Reuil, France). For ORN recordings from the pheromone sensilla in *S. littoralis*, we used the major pheromone component (Ljungberg et al., 1993), (Z)-9 (E)-11 tetradecadienyl acetate (Z9,E11-14:Ac; synthesized in the laboratory, courtesy of Martine Lettere).

2.2.2. Volatile plant compounds

Heptanal (98% purity, CAS 66-25-1) and a selection of VPCs belonging to different chemical families (aldehydes, acetates, terpenes) were used for some experiments: (Z)-3-hexenyl acetate (98% purity, CAS 3681-71-8), hexanal (>99% purity, CAS 66-25-1), octanal (98% purity, CAS 124-30-0), linalool (97% purity, CAS 78-70-6), geraniol (96% purity, CAS 106-24-1), geranyl acetate (mixture of isomers, >97% purity, CAS 16409-44-2), linalyl acetate (97% purity, CAS 115-95-7) and isoprene (> 98% purity, CAS 78-79-5). Mineral oil (CAS 8042-47-5) was used to prepare volume-to-volume dilutions at 0.1% and 1%. All compounds were purchased from Sigma Aldrich (Sigma Aldrich, Saint-Quentin Fallavier, France).

2.3. Olfactory stimulation

Odorants were delivered as described previously (Rouyar et al., 2011). Briefly, charcoal-filtered air was re-humidified and divided into eight equal flows (220 ml/min) directed each to a three-way miniature valve (the Lee Company, Westbrook-CT, USA). From there the flow could be directed to one 4 ml glass vial containing the stimulus source by activating the appropriate valve. The connections to the vials were made using PTFE tubing (1.32 mm ID) and hypodermic needles (18 G size). For practical reasons, due to their differences in volatility and polarity it was not possible to use the same type of stimulus sources for pheromone and VPCs. For VPCs, the vial contained 1 ml of solution in mineral oil at the appropriate concentration vol/vol. For the sex pheromone, the vial contained a section of PFTE tubing (1.6 mm ID; $L = 20$ mm) directly connected to a hypodermic needle and containing 10 or 100 ng of the sex pheromone blend. Stimulus- and clean air-carrying tubes were maintained together in a 10 cm long metal tubing constituting the stimulation pencil. A plastic cone of a P1000 pipette fixed at the output of the stimulation pencil served as a mixing chamber, approx. 5 mm in front of one of the moths' antennae, and focused on antennal sensilla. Programming of the electric valves was performed using a Valve Bank (AutoMate Scientific, Berkeley, USA) synchronized with the PC acquisition software.

In the different experiments, neurons were stimulated by individual pheromone pulses (from 0.1 to 0.5 s) or by a series of pheromone pulses in a VPC background (either 2.5 or 3 s long application starting 1 s before the pheromone pulse) compared to a clean air background, or a brief VPC pulse was provided in a pheromone background.

2.4. Electrophysiology

Males were briefly anesthetized with CO_2 and restrained in a Styrofoam holder. One antenna was fixed with adhesive tape. Single sensillum recordings were performed with electrolytically sharpened tungsten wires or glass microelectrodes. The reference electrode was inserted either in the abdomen, or in an antennal flagellomere a few mm from the flagellomere carrying the sensilla that we recorded from. The recording electrode was inserted into the base of a long trichoid hair situated on antennal branches of *A. ipsilon*, or on the edge of the antennal stem in *S. littoralis*. Recording and reference electrodes were connected to a Neurolog preamplifier (Digitimer, Hertfordshire, UK). The signal was filtered (0.2–10 kHz) and amplified 1000 times. The electrophysiological activity was sampled at 10 kHz and 12 bit resolution with a Data Translation DT3001 analogue to digital card. Signals were monitored on the computer screen using Awave software (Marion-Poll, 1995). For analysis, spike sorting and extraction of spike occurrence times from the recordings were also done using Awave software. The majority of our recordings showed the firing activity of only one ORN. When the activities of two neurons were recorded simultaneously in some of the long trichoid hairs housing Phe-ORNs (Fig. 1A), spikes were sorted by their amplitudes to analyze only the firing that showed changes in response to the sex pheromone.

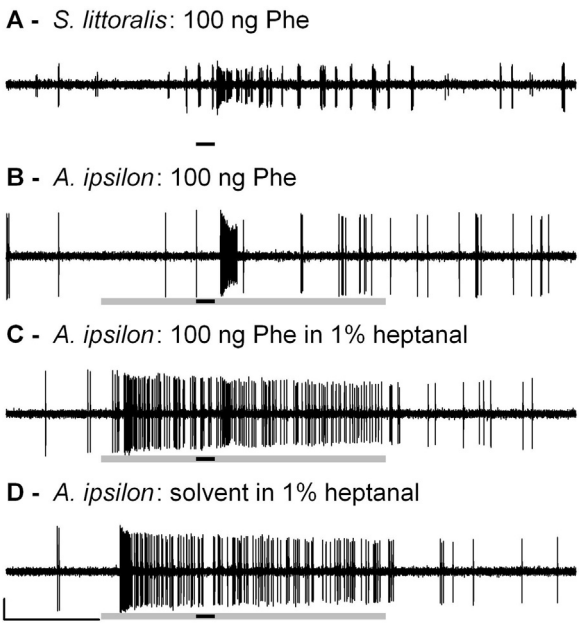

Fig. 1. Typical examples of extracellular recordings obtained from the long trichoid sensilla housing the Phe-ORNs in *Spodoptera littoralis* and *Agrotis ipsilon*. The recording sample in *S. littoralis* (A) shows the firing activity of two neurons, but only the neuron with the larger spikes responded to a pulse of 100 ng of Z9,E11-14:Ac, the main pheromone component. The Phe-ORN of *A. ipsilon* responded both to a short pulse of the pheromone (blend of Z7-12:OAc, Z9-14:OAc, Z11-16:OAc at a 4:1:4 ratio, 100 ng; (B) and to a long presentation of heptanal (D). The response to heptanal masked the pheromone pulse (C). Scale: vertical bar = 1 mV; horizontal bar = 1 s. The short horizontal black bar under the recordings indicates the pheromone stimulation (A–C; 0.2 s) or a solvent presentation (D). The long horizontal gray bars indicate the presentation of mineral oil as a control (B) or heptanal (C and D) (3 s).

2.5. Firing activity analyses

After extraction of spike occurrence times, firing rates were calculated using the local slope of the cumulative function of spike times (Blejec, 2005) using various R packages and custom-written R scripts (http://www.r-project.org/) (R Core Team, 2013). The slope was calculated over a moving spike window between the $n-2$ and $n+2$ spikes (five spikes). Thus, each spike was attributed a firing rate and its occurrence time. The maximum firing rate during the 1st second from stimulus start was measured for each recording. The mean ± standard error of the maximum firing rates were calculated for each stimulation and compared using a Student's t test for paired data followed by tests to check for data set normality (Shapiro test) and variance homogeneity (Fisher–Snedecor test).

The experimental decline of the averaged responses was fitted with an exponential asymptotic decay function by determining the non-linear least-squares estimates of parameter of an exponential model (function nls of *R*). Curves of firing rates were standardized relatively to the maximum firing rate. The asymptotic decay functions were estimated from the time of the maximum firing rate to 3.5 s after the onset of stimulation:

$$FR = a + b * e^{(-c*\text{time})}$$

where FR is the maximum firing rate, a is the offset, b the initial firing rate, and c the rate coefficient of the curve. The time values for 95% decay (td_{95}) were calculated from this equation. Then, we performed a bootstrap procedure ($n_b = 1000$) over observed data in non-odorized air or linalool-odorized air and calculated the td_{95} for each random sample and their 95% confidence interval. Estimations of mean and variance for the bootstrapped samples were used to test the null hypothesis (presence of background did not affect td_{95}; t-statistics).

Table 1
Percentage of reduction of the firing response of Phe-ORNs to a pulse of 1 μg of Z9,E11-14:Ac in the presence of a background of one VPC (at 1% dilution in mineral oil). Data present the relative decrease of mean firing rate measured during pheromone presentation in the presence of the background compared to the response in non-odorized air.

Compounds	% Reduction	*N*
Geraniol	58.0	19
Linalool	44.6	12
Geranyl acetate	34.6	19
Linalyl acetate	32.9	20
cis-3-Hexenyl acetate	2.5	26
Isoprene	2.3	18

We estimated the response latency for each recording using custom-written R scripts. First, we calculated a threshold for excitation response as the 95th percentile of spike firing rates before stimulation onset (spontaneous activity). Second, we looked for the first spike crossing this threshold within the expected response time window corresponding to 1 s after stimulation start. We defined this spike occurrence time as response latency. Survival functions of the latency data were estimated using the Surv( ) R function. We compared median latencies between two treatments using their Kaplan–Meier plots and a chi-square test.

The quartile coefficient of dispersion was calculated as $(Q_3 - Q_1)$/median after calculation of the quartile values with the function boxplot from R package graphics.

3. Results

3.1. VPCs alter intensity coding in the pheromone subsystem

In *S. littoralis* a background of linalool (at 1% dilution in mineral oil) considerably modified the response of Phe-ORNs to a single pulse of the main pheromone component Z9,E11-14:Ac (Fig. 2A). A 0.5 s pulse of 1 μg of Z9,E11-14:Ac in non-odorized air elicited a strong and quick increase in firing activity peaking at 200 spikes/s followed by a slower decrease of firing frequency (Fig. 2, A1). A linalool background alone applied during 2.5 s did not activate the firing of Phe-ORNs (Fig. 2, A2). The maximum firing frequency in response to Z9,E11-14:Ac was considerably reduced in the presence of the linalool background (Fig. 2, A3). Note that at the end of application of linalool the firing activity presented a positive rebound (arrow, Fig. 2, A3). The dose response curve for the pheromone was shifted to the right in the presence of linalool, indicating a reduction of sensitivity to the pheromone (Fig. 2B). The maximum response was also lowered, revealing a reduction of efficacy. Both effects indicate a strong degradation of the capacity of Phe-ORNs to code the intensity of the pheromone signal.

We also tested several other VPCs: linalool, geranyl acetate, linalyl acetate, geraniol, cis-3-hexenyl acetate, and isoprene, for their effects on pheromone detection in *S. littoralis*. The response to a 0.5 s pulse of Z9,E11-14:Ac at 1 μg was measured within a 2.5 s long puff of VPC. The four terpenic compounds reduced the response to the pheromone pulses, geraniol and linalool being the most potent inhibitors (Table 1). In turn, the two non-terpenic compounds, isoprene and cis-3 hexenyl acetate did not reduce the response.

Sex pheromone is a very strong stimulus for male moths engaged in mate searching that may even ignore predator signals they would normally avoid (Skals, 2005; Skals et al., 2003). This risky behavior enables them to keep flying toward low concentrations of pheromone even in the presence of stronger other sensory cues. However, the pheromone salience could be significantly affected by changes in the intensity of sensory inputs. Still, confronted to an odorant background the relative increase in firing

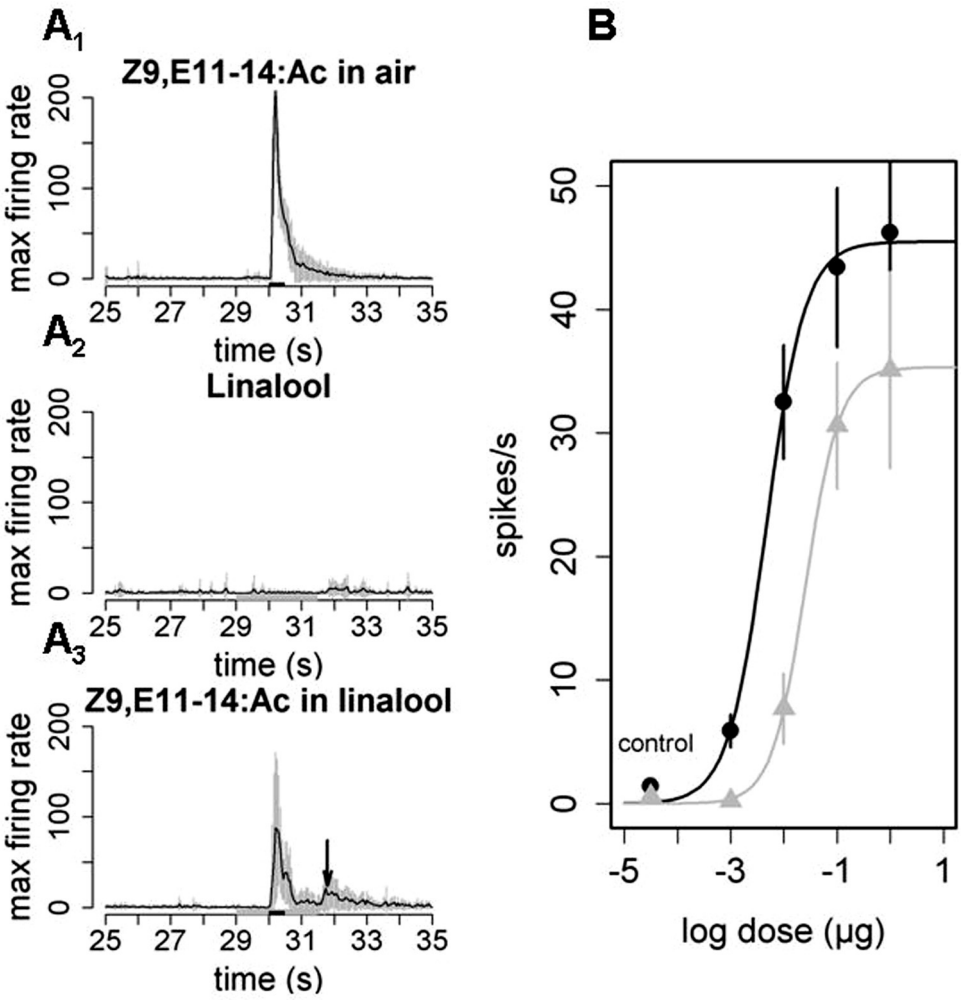

Fig. 2. A linalool background reduces the amplitude of the response of Phe-ORNs to a pulse of pheromone in *Spodoptera littoralis*. (A) Maximum firing frequency plots of responses to a 0.5 s pulse of Z9,E11-14:Ac (black bar) at 1 μg in non-odorized background (A_1),or in a background of linalool (A_3, during 2.5 s using a source of linalool diluted at 1% (v/v) in mineral oil; gray bar). The vertical arrow indicates the rebound in firing at the end of linalool presentation. Linalool background itself (A_2) did not stimulate the Phe-ORNs. Means of $N = 17$ recordings calculated over successive time windows of 0.05 s and standard deviation (bars in gray). (B) Dose response curves, mean number of spikes ($N = 12–17$). Black = Z9,E11-14:Ac in non-odorized air; gray = Z9,E11-14:Ac in linalool-odorized air. Error bars = standard deviation.

due to pheromone might be more important than its absolute intensity. We thus tested whether a reduction of the absolute firing intensity in response to pheromone pulses changed the prominence of the pheromone response above the random activity in a sample of Phe-ORNs. In the absence of pheromone stimulation the spontaneous firing activity of individual Phe-ORNs was low and at random. In response to a pheromone pulse (Figs. 1 and 2, from 30.0 to 30.5 s) the firing frequency increased considerably during the pulse in all the individual neurons. We calculated the ratio of the sample mean firing activity to its standard deviation (the reciprocal of the coefficient of variation, $CV^{-1} = \mu/SD$, where μ is the estimated mean and SD is the estimated standard deviation of the firing activity) by time windows and expressed it as a function of time. This ratio provides an estimate of global signal to noise ratio (SNR) for the response to a pheromone pulse and a quantification of the capacity of the population of neurons to encode a sudden increase in pheromone concentration. At the level of the neuron sample, the mean firing intensity increased relatively more than the inter-neuron variability, so the pheromone pulse was translated into a relative increase in the net global output. Thus, comparing the evolution in time of CV^{-1} under the different odor backgrounds enabled us to evaluate the negative effects of a VPC background on the encoding of pheromone pulses by the pheromone sub-system. Plots of CV^{-1}, as a function of time, show that linalool, geraniol, linalyl acetate and geranyl acetate all strongly reduced the peak of CV^{-1} (Fig. 3). Furthermore the CV^{-1} increased again when the background was turned off (at 31.5 s), a phenomenon that could further complicate the extraction of the information relevant to the pulse of pheromone (Fig. 3, indicated by arrow). In turn, neither cis-3-hexenyl acetate nor isoprene affected the signal to noise ratio of the pheromone pulse probably because both compounds neither activated the Phe-ORNs by themselves, nor showed competitive effects when applied together with pheromone.

3.2. *VPCs stimulate the Phe-ORNs of* A. ipsilon *and affect quality coding*

Contrary to *S. littoralis*, in which VPCs did not excite the Phe-ORNs, in *A. ipsilon* linalool, (Z)-3-hexenyl acetate (Z3-6:Ac), hexanal and even more, heptanal stimulated the firing activity of Phe-ORNs (Fig. 4, middle column). The Phe-ORNs responded in a phasic-tonic mode to a 3-s stimulation with these VPCs. Consequently, the response to a short pulse of pheromone (Fig. 4, right column) was, at least partially, masked by the 3-s VPC stimulus. With heptanal the masking was almost complete. Thus, the presence of a VPC can

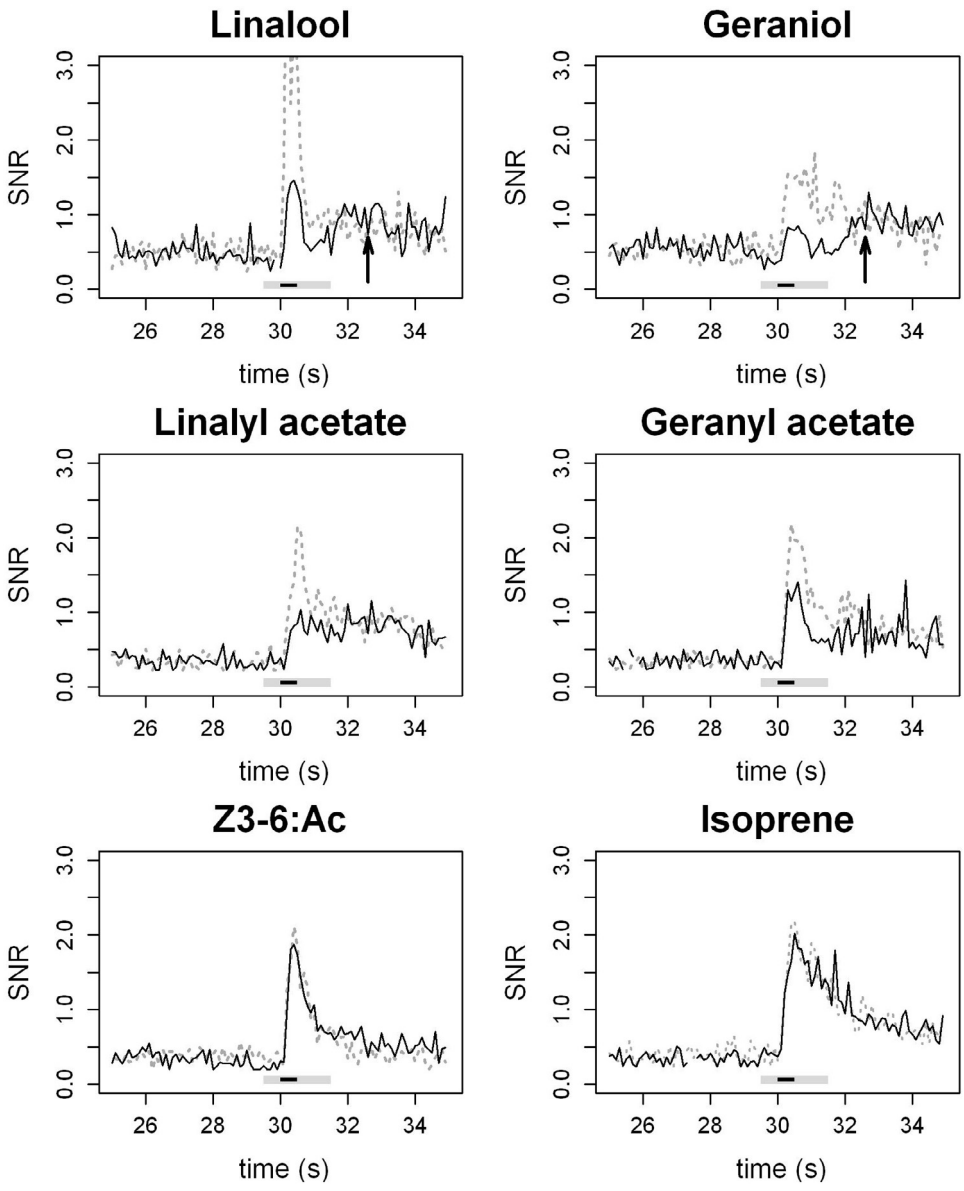

Fig. 3. A VPC background affects the coding of a pheromone pulse in a population of Phe-ORNs in *S. littoralis*. The plots present the signal to noise ratio (CV^{-1} = mean/standard deviation) for the maximum firing rates over successive time windows of 0.1 s each (numbers of recordings varied according to treatment: linalool $N = 17$, geraniol $N = 18$, linalyl acetate $N = 20$, geranyl acetate $N = 19$, Z3-6:Ac $N = 26$, isoprene $N = 18$). Stimulus marks are presented under the curves: pheromone (1 μg of Z9,E11-14:Ac), pulse of 0.5 s (black bar); VPC concentration = 1% in mineral oil, during 3.5 s (light-gray bar). Dashed gray line = response in non-odorized air; solid black line = response in VPC-odorized air. An arrow indicates the rebound following the background offset when present.

fallaciously increase the sensory input to the pheromone subsystem and alter its ability to discriminate the pheromone signal from the odorant background.

3.3. VPCs affect coding of pheromone signal temporality

We designed experiments in order to determine how antagonistic or agonistic activities of VPCs on Phe-ORNs could affect the dynamic coding of the temporal parameters of the pheromone signal. We tested two different situations: the coding of a sustained pheromone signal and the coding of the duration of a single isolated pheromone pulse.

First, we stimulated the Phe-ORNs of *S. littoralis* with a 2.5-s pheromone stimulus and applied a 0.5 s pulse of linalool in the middle of the stimulation period, when the neurons were firing (Fig. 5). The Phe-ORNs responded in a phasic-tonic mode to a long pheromone stimulus. When the short linalool pulse was applied 1 s after the start of the pheromone pulse, the firing activity was decreased down to its spontaneous activity level (Fig. 5).

Second, we analyzed the effects of a background of linalool on the dynamics of the response to a 0.5 s pulse of pheromone in Phe-ORNs of *S. littoralis*. These experiments confirmed that linalool reduced the amplitude of the firing response of Phe-ORNs to Z9,E11-14:Ac (Fig. 6A). The analysis of the latency revealed that the Phe-ORN response was delayed in the presence of linalool (0.2 s in linalool versus 0.17 s in non-odorized air, $Chi^2 = 8.1$, one degree of freedom, $P = 0.0045$; Fig. 6B). Finally, the model decay curves were different with a shorter response decay ($td_{95} = 0.653$ s) in linalool-odorized air, compared to non-odorized air ($td_{95} = 0.727$ s; Fig. 6C), the *t*-statistics confirming this difference was significant ($z = 3.12$, $P = 0.0018$). Note the presence of a rebound in the firing activity at the end of the application of the linalool background (at $t = 1.5$ in Fig. 6C, right).

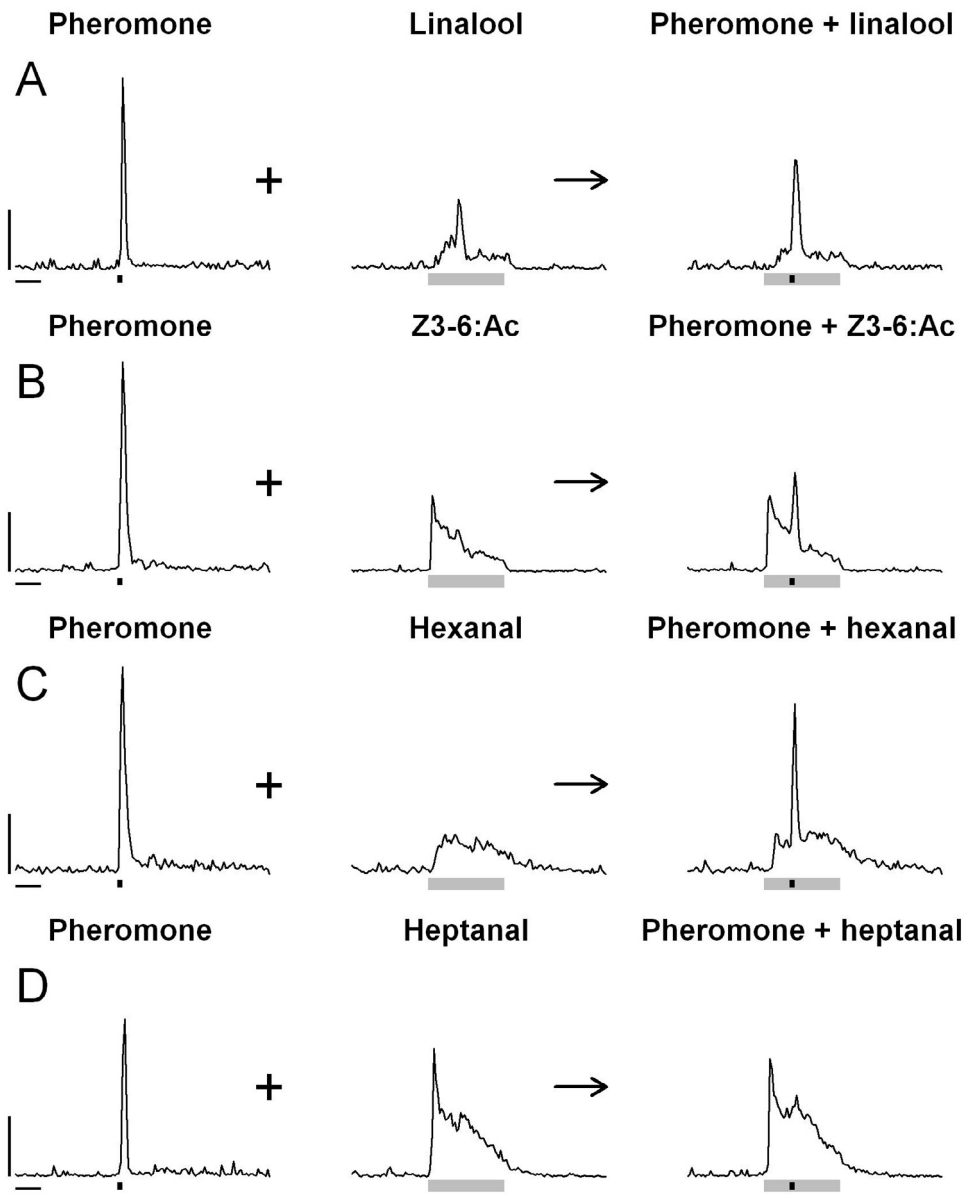

Fig. 4. The Phe-ORNs of male *Agrotis ipsilon* are excited by volatile plant compounds. Frequency plots averaged from responses recorded from 14 to 19 different sensilla (means of maximum frequency over bins of 0.075 s; $N = 19$ for linalool (A), Z3-6:Ac (B), and hexanal (C), $N = 14$ for heptanal (D)). Black bars = pheromone stimulation (blend of Z7-12:OAc, Z9-14:OAc, Z11-16:OAc at a 4:1:4 ratio, 100 ng, 0.5 s pulse); gray bars = VPC stimulation (solution 1% (v/v) in mineral oil, 3 s presentation). Horizontal axis: time, scale bar 1 s; vertical axis: maximum firing rate, scale bar: 50 spikes/s.

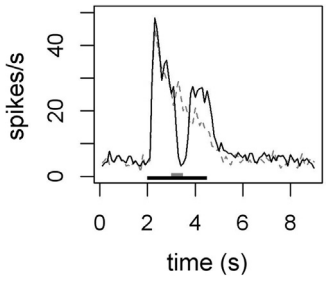

Fig. 5. A pulse of linalool interrupts the response to a prolonged pheromone stimulus in *S. littoralis*. A 0.5 s pulse of linalool (1% dilution in mineral oil, gray bar) temporally inhibits the firing of Phe-ORNs in response to a long pheromone stimulus (100 ng of Z9,E11-14:Ac, 2.5 s – black bar). Mean firing rates ($N = 22$), bin size = 0.1 s. By contrast to the response to pheromone only (dashed line), note a rebound in firing activity during the response to the prolonged pheromone stimulus at the end of the linalool pulse (solid line).

3.4. VPCs compromise the reproducibility of the response

We observed throughout the preceding experiments that not all Phe-ORN responses were equally affected by the presence of an odorant background. Consequently, the dispersion of the response intensity was generally greater in the presence of a VPC background compared to non-odorized air (compare for instance the size of SD bars on A1 and A3 in Fig. 2). Thus we show below a more detailed analysis of data dispersion.

As expected, the median of the maximum firing rate in response to the pheromone blend was lower in a background of heptanal (Fig. 7A) in *A. ipsilon* and the total number of spikes fired during the second following the stimulus onset increased (Fig. 7B). In turn, the time of peak was slightly advanced (Fig. 7C). The increase in the total number of spikes is probably directly connected to the fact that heptanal excites the Phe-ORNs of *A. ipsilon*. With respect to data dispersion, the quartile coefficient of dispersion was increased for

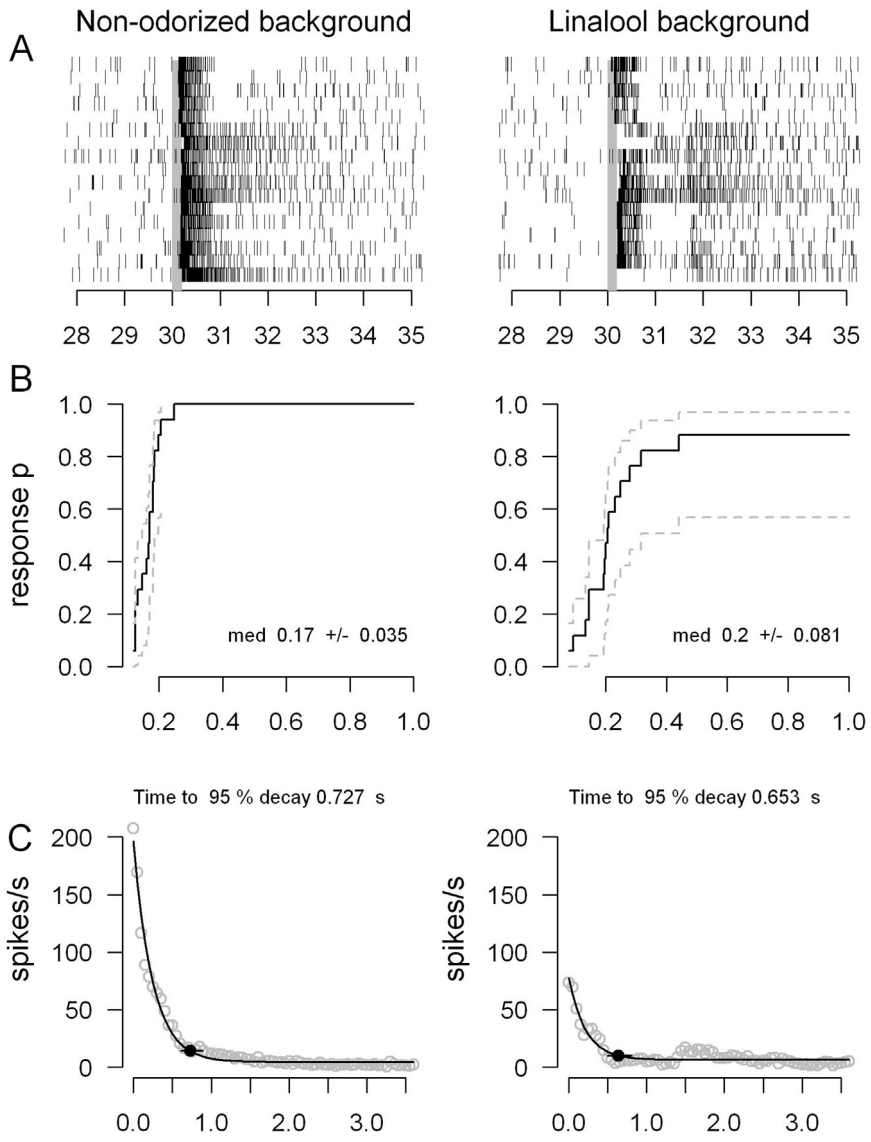

Fig. 6. Effects of linalool on the response dynamics of *S. littoralis* Phe-ORNs to a 0.2 s pulse of pheromone. Left column: responses to pheromone in non-odorized air; right column: responses to pheromone in 1% linalool ($N = 17$). Horizontal axes are time in s for all graphs; vertical axes different units. (A) Raster plots of responses of individual neurons; the vertical gray bar indicates the pheromone pulse. (B) Kaplan–Meier curves of the response latencies; p is the proportion of neurons that responded to the olfactory stimulus. (C) Exponential decrease model for response end. The open circles present the experimental values, the solid line shows the estimated exponential decrease and the large black dot is the estimated value for 95% decrease with a 95% confidence interval (horizontal line).

maximum firing rate, number of spikes and peak time in *A. ipsilon* and *S. littoralis* (see corresponding values in Fig. 7), indicating an increase in inter-neuron variability in the static and dynamic components of the response to pheromone. The increase of dispersion of time of peak firing in both species indicates that the phasic components of the firing activity of Phe-ORNs are less synchronized in the presence of an odorant background. In *S. littoralis*, increases in the dispersion of the measures of maximum firing rate and peak time were also observed in the presence of a linalool background. It should be noted that in this case the phasic component of the response was delayed.

Globally this analysis confirmed that the introduction of a VPC background increased the inter-neuron variability, with some specificity in the response parameters that were most affected with respect to the moth species and the type of activity of the compound (antagonism for linalool in *S. littoralis*, partial agonism for heptanal in *A. ipsilon*). How these dispersion effects may contribute to altering the coding of the pheromone signal is still to be investigated. One possible issue is that it can alter the consistency of activity maps in Phe-ORNs.

To check this hypothesis we constructed activity maps from series of recordings and compared activity maps obtained in non-odorized versus odorized air (Fig. 8). A population of $N = 33$ Phe-ORNs, sampled on different antennae, were submitted to a train of 10 pulses (0.05 s) of Z9,E11-14:Ac in linalool-odorized and non-odorized air. The maximum firing rate reached during each pheromone pulse was measured. Two matrices of 10×33 were obtained and used to draw activity maps where a line presented the activity measured in the different cells for the same pulse number, and a column the activity of the same cell in response to the 10 subsequent pulses, and a 15-gray-level code the frequency of the firing. The distribution of activity remained consistent among neurons between pulses, although the response decreased in intensity (Fig. 8) in both background conditions. However, it changed between responses in non-odorized air versus linalool odorized air suggesting that the background, by changing the distribution of the

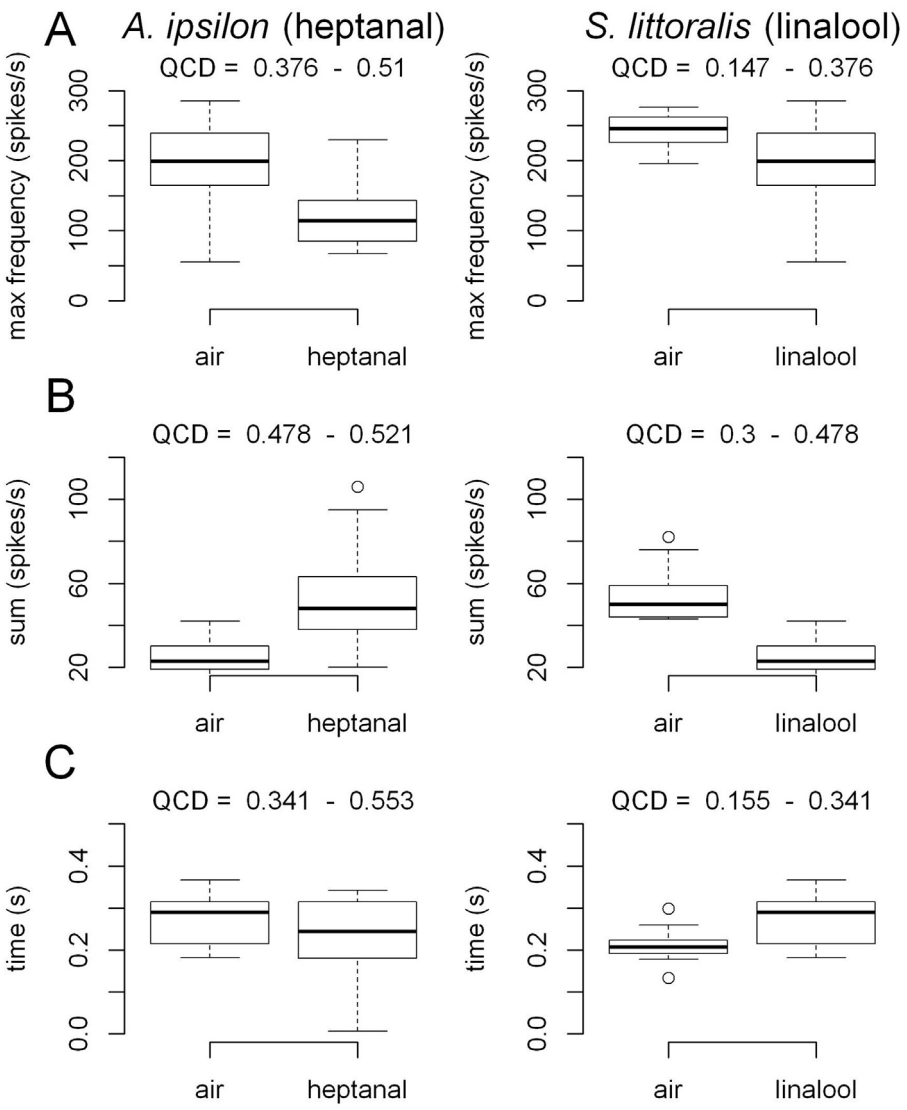

Fig. 7. A background of plant volatile increases the dispersion of the response parameters. Box plots for three different parameters of the firing response of Phe-ORNs to pheromone: (A) maximum firing rate; (B) number of spikes during 1 s; (C) time of peak of firing activity. Parameters were measured on a time window between $t = 30$ s and $t = 31$ s in *S. littoralis* ($N = 17$ Phe-ORNs) and *A. ipsilon* ($N = 25$ Phe-ORNs). The lower whisker presents the minimum, the lower hinge of the box is the first quartile, the line inside the box is the median, the upper hinge is the third quartile, and the extreme of the upper whisker is the maximum; outlier values are indicated by a circle. QCD, quartile coefficient of dispersion.

input might affect the coding of the pheromone signal. In particular, four neurons that did respond (numbers 6, 26, 28 and 31) to the 10 pheromone pulses in non-odorized air, did not respond in linalool-odorized air; in turn three other neurons (numbers 8, 20, 29) did neither respond in non-odorized nor linalool odorized air.

4. Discussion

Our results show that the coding of intensity, quality and temporality of the highly specific sex pheromone signal in moth ORNs is modified by a background of plant volatiles. These findings confirm and extend earlier work with simpler or similar stimulation protocols in the same two species (Deisig et al., 2012, 2014; Party et al., 2009; Rouyar et al., 2011). In addition, we reveal here that a background of VPCs increases the already naturally present heterogeneity of the different elements in the firing response to the sex pheromone. This effect concerned both investigated species and different general odorants.

There might be both experimental and biological origins of the heterogeneity in Phe-ORN responses. Experimentally, variability in response to the pheromone could result from the conditions of stimulation, but measurements with a photoionization detector have shown that signals are highly repeatable in laboratory conditions very close to ours (Rospars et al., 2014). A second source of variability is the heterogeneity in the odor flows or position of sensilla relative to the stimulus flow. Such variations in the exposure of Phe-ORN to the stimulus are expected to also occur in natural conditions. Once released by female moths, the sex pheromone is transported by turbulent air flows to form a meandering plume comprised of odor filaments intermingling with non-odorized air pockets (for a description of the distribution of odors under turbulent air regime see Celani et al., 2014). When such a filament hits the male antennae, the pheromone molecules reach some of the Phe-ORNs housed in sensilla distributed all over the antenna length whose dendritic membrane carries the olfactory receptors (ORs). Thus, in the laboratory as in the field, Phe-ORNs from remote sensilla will be differently exposed to pheromone molecules and their response will vary accordingly. In addition to this experimental variability, also differences in receptor–ligand interactions, the density of ORs on the ORN dendrites, or differences in ion

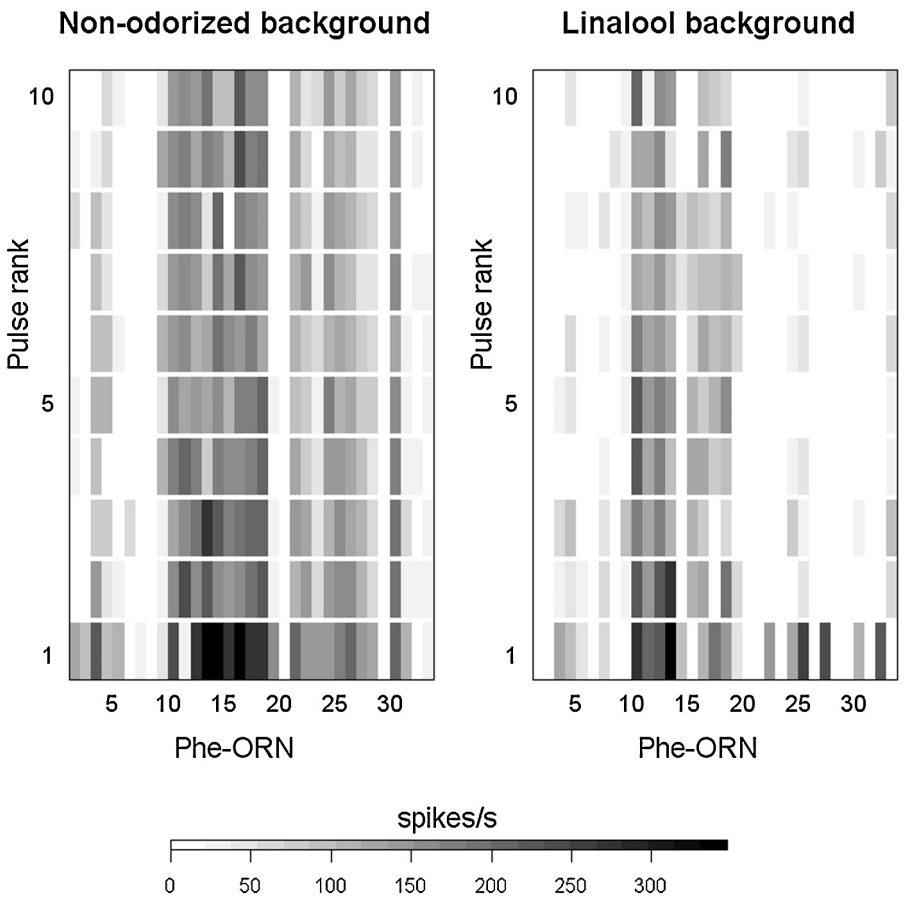

Fig. 8. A linalool background changes the activity map through a sample of Phe-ORNs in *S. littoralis*. $N = 33$ Phe-ORNs were submitted to a train of 10 pulses (0.05 s) of Z9,E11-14:Ac. The maximum firing rate reached for each pulse was calculated. Distributions of the activity of neurons 1–33 (*x* axis) and pulse rank (1–10, *y* axis) are displayed in activity maps where a line presents the activity measured in the different neurons for the same pulse number, and a column the activity of the same neuron in response to the 10 subsequent pulses. While the distribution of activity remained consistent between pulses, it changed between responses in non-odorized air versus linalool-odorized air.

channels involved in the transduction process could also contribute to inter-cell variability (Jacquin-Joly and Lucas, 2005). Consequently, variability at the periphery is an inherent natural component of the olfactory system so that central neurons are expected to receive a highly variable input from the tens of thousands Phe-ORNs present in the moth antennae.

Insect olfactory receptors present different degrees of chemical tuning (Andersson et al., 2015). The narrow response spectrum of the pheromone receptors limits the influence of general odorants on pheromone detection. However, this chemical specificity has some limits. A volatile plant compound, linalool, has been shown to interfere directly with the binding of the main pheromone component to its specific olfactory receptors in *Heliothis virescens* (Pregitzer et al., 2012). A male moth flying upwind while following a pheromone plume will be exposed to variable levels of potentially interfering volatiles when crossing areas odorized with general odorants released by the different plant communities it will travel through. Adding to the variability for pheromone stimulation alone, this changing and intense VPC background could influence heterogeneity in pheromone responses in several ways. First agonistic or antagonistic interactions with pheromone receptors will locally alter the perception of pheromone concentrations. Second, since an odorant background, whatever its effects on pheromone detection, is expected to reach the ORNs with a different time-course from that of the specific signal, variations in time-lapse between the two odors are expected to increase the variability of the final response. Our postulate was that not only the presence of VPCs would alter the transduction of the pheromone signal into spike trains, but that it would also add a supplementary factor of variability and affect the consistency of the detection of the pheromone signal, variability being expected to increase with complexity of the sensory environment (increase in the degrees of freedom). Shifted dose response curves of *S. littoralis* Phe-ORNs to pheromone in the presence of a linalool background confirmed that the sensitivity may be reduced and coding of absolute pheromone concentration may be altered by a VPC background. However, it is probably more important for the male moth to be able to extract short firing events from the whole sensory input as they reveal the presence of a pheromone filament. We observed that in the presence of terpenes the responses to a single pulse of pheromone became less visible over the firing activity in our sample of Phe-ORNs and this was confirmed by analysis of the signal to noise ratios. This finding indicates that a reduction of salience occurs already at the sensory input in the pheromone sub-system, for which the respective contribution of decreased sensitivity and increased inter-neuron variability must be evaluated.

Intensity coding can be challenged in another way than antagonism. In *A. ipsilon*, heptanal stimulated the firing activity of Phe-ORNs while reducing the response to a pulse of pheromone. In this insect heptanal is a partial agonist of the pheromone, able to activate the Phe-ORN by itself and producing mixture suppression when mixed to the sex pheromone. This loss of specificity indicates that quality coding is also affected, in spite of the chemical specificity of the Phe-ORNs. Furthermore, inside a linden canopy,

male moths will be permanently exposed to high atmospheric levels of heptanal which should partially adapt their Phe-ORNs with reduced sensitivity as a possible consequence. Finally, either antagonism or partial agonism, by reducing the response, resulted in masking of the pheromone pulse. It has been postulated that the filamentous nature of the pheromone plume may render the pheromone signals relatively protected from background chemical noise like the pheromone of other insect species (Liu and Haynes, 1992), because, although pheromone plumes of different species may overlap spatially, it is less likely that individual pheromone filaments within overlapping plumes will mix to a great extent. However, behavioral inhibitors present in the pheromone blends of sympatric moth species are detected through channels (types of ORNs), separated from those of intraspecific pheromone components (Todd and Baker, 1999), thus potential interactions are different from our case, where a VPC background interferes directly with the pheromone detection channel. The stochastic intrinsic nature of the interactions between a ligand molecule and the olfactory receptor contribute to variability in response among neurons that may be further increased by random interferences with "non-signal" odorants. The comparison of our recordings in non-odorized versus odorized air confirmed an increase in the dispersion of the measures of the firing responses of Phe-ORNs in odorized conditions. This populational effect concerned two species and different VPCs. It resulted in increasing the fuzziness of signal extraction, an identical stimulus producing variable patterns of activity. The importance of these variations to pheromone coding deserves to be more deeply investigated since it might affect not only intensity coding, but could also be critical for quality coding when fine ratio discrimination is required.

The pheromone system of moths is characterized by a very large number of Phe-ORNs converging onto a far smaller number of central neurons in the antennal lobes (Hansson and Anton, 2000). Central neurons might operate a kind of "smoothing" or "averaging" of neuronal input (e.g. Galizia and Szyszka, 2008; Wilson et al., 2004) but alternatively, stable differences of the relative activity between ORNs might provide relevant information on the evolution in space and time of the pheromone signal (Rospars et al., 2014). Viewing the data as ORN activity maps clearly showed that the presence of an odorant background changed the distribution of antennal neurons being active in response to pheromone pulses. How central neurons in the first olfactory center, the antennal lobe, might use the variability in ORN input to improve coding of relevant olfactory information is currently under investigation. Central neurons might average the neuronal input, but alternatively, stable differences of the relative activity between ORNs might be preserved and provide relevant information on the evolution in space and time of the pheromone signal. The antennal lobe network transforms ORN information in a non-linear way, amplifying weak inputs at the projection neuron level and redistributing information in between glomeruli (Bhandawat et al., 2007). A recent study in *A. ipsilon* has revealed that the heterogeneity of ORN responses to the sex pheromone contributes to the high sensitivity and fast responses of projection neurons in the antennal lobe (Rospars et al., 2014). For certain types of pheromone-responding antennal lobe neurons, it has been shown that addition of a VPC to the sex pheromone (simultaneous stimulation with both) can indeed improve the resolution of pulsed pheromone stimuli (Chaffiol et al., 2012).

The VPCs used for this study are naturally present in the environment of moths and produced in significant amounts by a number of plants, some of which being oviposition sites or food sources for the two moth species investigated. The concentrations used in our study were rather high, with respect to their emission rates in the field. Nevertheless, our experiments point out that in spite of the narrow chemical tuning of the pheromone sub-system, the detection of a pheromone signal may be altered by the presence of VPCs in the air. Models for the evolution of our environment due to global change predict increasing concentrations of volatile organic compounds (VOCs) in the troposphere, notably because of the climatic stress caused to plants (Peñuelas and Staudt, 2010) and the increase in VOCs of anthropogenic origin. This fast change in the odor environment might cause a major stress to biodiversity by perturbing olfaction of pollinators or pest insects. Understanding more deeply the effects of complex and variable odor backgrounds on insect olfaction is thus of increasing importance.

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