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Measuring activity in olfactory receptor neurons in *Drosophila*: Focus on spike amplitude

Fernando Martin, Esther Alcorta *

Department of Functional Biology, Faculty of Medicine, University of Oviedo, Oviedo, Spain

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ABSTRACT

Olfactory responses at the receptor level have been thoroughly described in *Drosophila melanogaster* by electrophysiological methods. Single sensilla recordings (SSRs) measure neuronal activity in intact individuals in response to odors. For sensilla that contain more than one olfactory receptor neuron (ORN), their different spontaneous spike amplitudes can distinguish each signal under resting conditions. However, activity is mainly described by spike frequency.

Some reports on ORN response dynamics studied two components in the olfactory responses of ORNs: a fast component that is reflected by the spike frequency and a slow component that is observed in the LFP (local field potential, the single sensillum counterpart of the electroantennogram, EAG). However, no apparent correlation was found between the two elements.

In this report, we show that odorant stimulation produces two different effects in the fast component, affecting spike frequency and spike amplitude. Spike amplitude clearly diminishes at the beginning of a response, but it recovers more slowly than spike frequency after stimulus cessation, suggesting that ORNs return to resting conditions long after they recover a normal spontaneous spike frequency. Moreover, spike amplitude recovery follows the same kinetics as the slow voltage component measured by the LFP, suggesting that both measures are connected.

These results were obtained in ab2 and ab3 sensilla in response to two odors at different concentrations. Both spike amplitude and LFP kinetics depend on odorant, concentration and neuron, suggesting that like the EAG they may reflect olfactory information.

1. Introduction

Olfactory responses at the receptor level have been thoroughly described in intact *Drosophila melanogaster* by electrophysiological methods. Two different types of extracellular measurements have been used to describe in vivo responses to odorants: field measurements of the olfactory organs, such as electroantennograms (EAGs) (Venard and Pichon, 1981; Borst, 1984; Alcorta, 1991; Gomez-Diaz et al., 2004, 2006) and electropalpograms (EPGs) (Ayer and Carlson, 1992; Riesgo-Escovar et al., 1994, 1997), and analysis of neuronal activity by evaluating spike frequency via single sensillum recording (SSR) (Clyne et al., 1997; de Bruyne et al., 1999, 2001). For single sensillum that contain more than one olfactory receptor neuron (ORN), different spike amplitudes distinguish each neuron's signal, especially under resting conditions. Firing rate analysis of the responses to many odorants has been successful in defining the response profiles of different ORNs (de Bruyne et al., 1999, 2001; Dobritsa et al., 2003; Hallem et al., 2004; Hallem and Carlson, 2006; Yao et al., 2005; Van der Goes van Naters and Carlson, 2007; Silbering et al., 2011) and has been used to address odorant concentration and duration coding (Hallem and Carlson, 2006). Since its implementation, this type of measurement has been extensively applied to describe ORN activity in response to odors and to elucidate the molecular mechanisms that are responsible for odor reception and information coding at the receptor level (Raman et al., 2010; Sargsyan et al., 2011; Martelli et al., 2013).

In a few cases research on olfactory population codes and the biophysical mechanisms underlying ORN response dynamics add to spike frequency analysis EAGs or local field potential measurements (LFPs) in single sensilla at the maxillary palps (Olsen et al., 2010; Nagel and Wilson, 2011). Using these methods, two different components of the olfactory responses of ORNs have been defined: an initial fast component that is reflected by spike firing, and a later slow component that is observed in the LFP response. Spike frequency and LFP kinetics exhibit no apparent correlation. However, both measurements reflect the status of the sensillum, which contains 1–4 ORNs, and its ability to respond to a stimulus.

All of these studies considered spikes as quantum measurements of ion exchange through the cellular membrane; as such, quantifying spiking rate can adequately describe neuronal activity. However, if the membrane potential changes in a more sustained manner as a consequence of odorant response, the equilibrium of the driving forces through ion channels could change, and spike amplitude could also be affected. Such a correlation between slow voltage changes (EOG, electro-olfactogram) and spike amplitudes in fibers of the olfactory nerve has been reported in a complex double-stimulation system involving electrical stimulation and odors in rats (Scott and Sherrill, 2008).

* Corresponding author at: Department of Functional Biology, Faculty of Medicine, University of Oviedo, Julian Claveria 6, 33006 Oviedo, Spain.
Email address: ealcorta@uniovi.es (E. Alcorta)

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We have exploited the special architecture of the *Drosophila* sensillum, which exhibits marked compartmentalization (Shanbhag et al., 1999, 2000), to explore this relationship at the single ORN level. In this report, we analyze the SSRs of ab2A and ab3A neurons, which are responsible for the largest spikes, in their corresponding sensilla in response to two odorants, ethyl acetate and pentyl acetate, at several concentrations. We test the time course of spike amplitudes under different conditions for odorants that evoke different response patterns either in the same ORN or in different ORNs. Thus, we measured responses to ethyl acetate and pentyl acetate, which evoke opposite responses in the ab2A neuron, typically excitatory and transient responses respectively, and to pentyl acetate in the ab3A neuron, where it elicits an excitatory response as ethyl acetate. By comparing spike frequency, spike amplitude and slow LFP in response to different stimulus concentrations, we also attempt to decipher how these parameters relate to each other during ORN signaling.

2. Materials and methods

2.1. Fly stocks

Drosophila melanogaster wild-type Canton-S flies used for analysis of native ab2 and ab3 sensilla were obtained from the Bloomington Stock Center (Bloomington, Indiana, USA). Recordings were restricted to adult 4–6 day old females. Females are used as representative of both sexes because no striking molecular or physiological sexual dimorphisms have yet been reported in the peripheral olfactory system in *Drosophila* in response to general odors (Benton and Dahanukar, 2011) an especially in the morphology of ab2 and ab3 sensilla (Shanbhag et al., 1999). The flies were grown at a temperature of 25 °C under a 12:12 h light-dark cycle.

To observe the responses of ab2A neurons only, we killed ab2B neurons by expressing diphtheria toxin light chain (named DT-A.I or DTI) under Gal4/UAS control (*w; UAS-Cbβ\DT-A.I/Or85a-Gal4; +,* in short *Or85a-Gal4/UAS-DTI*), as described for the olfactory system in Schlief and Wilson, 2007; Silbering et al., 2011; Grosjean et al., 2011. Thus, by expressing DTI in cells expressing the Or85a receptor (i.e., the ab2B neurons), we obtained ab2-modified sensilla containing only the ab2A neuron. Depletion of the ab2B neuron was confirmed in each tested fly by the absence of the small spontaneous spike in the SSR leaving only the big spontaneous spike. The *w; P{UAS-Cbβ \DT-A.I}18/CyO* and *w; P{Or85a-GAL4.F}67.2* stocks for generating heterozygous Gal4/UAS flies were obtained from the Bloomington Stock Center (Bloomington, Indiana, USA) and were donated by Leslie Stevens and Leslie Vosshall, respectively.

For SSR recordings, only 1 sensillum was tested for each fly. This sensillum was measured for response to three concentrations of ethyl acetate and pentyl acetate. Different flies were used for recording from ab2 and ab3 sensilla. Different flies were used for LFP recordings and EAGs.

2.2. Single sensillum recordings (SSR)

SSRs were performed in flies mounted in truncated pipette tips according to previous protocols (Martin et al., 2011; Benton and Dahanukar, 2011). In short, the pipette tip was fixed with wax on a microscope slide, and the antennae was gently placed on a cover slip and stabilized with a glass micropipette (method modified from Clyne et al., 1997). Action potentials were recorded by inserting a naked tungsten wire electrode in a sensillum. The tip of the electrode was thereby brought in contact with the sensillum lymph surrounding the dendrites of the ORNs. The tungsten wire (0.25 mm diameter) was electrolytically sharpened (ca. 1 μm tip diameter) by repeated dipping in a 20% $NaNO_2$ solution while passing a 5 A current through the solution. The reference electrode was inserted at the eye or the base of the proboscis.

Electrical signals were acquired using a Universal Single-Ended Probe (Syntech, Hilversum, The Netherlands) and were filtered, amplified and recorded at 96 kHz with an IDAC 4 data acquisition recorder (Syntech, Hilversum, The Netherlands). Electrical signals were recorded for 10 s, beginning 1 s prior to stimulation.

For spike waveform recording, AC signals (100–10,000 Hz) were acquired. We identified ab2 and ab3 sensilla according to their positions and functional profiles (de Bruyne et al., 2001). The activities of co-located olfactory receptor neurons (ORNs) in ab2 and ab3 sensilla were distinguished whenever possible based on differences in spike amplitude and shape. The ORN with the largest spike amplitude was termed A; the second largest was termed B.

For Local Field Potential recording (LFP), DC signals were acquired and filtered using the IDAC-4 built-in EAG filter, a 5th order 12 Hz low pass filter with Bessel characteristics (DC-12 Hz).

2.3. Electroantennogram recordings (EAGs)

EAGs were performed in flies mounted in truncated pipette tips, as described by Alcorta (1991). Electrical activity was recorded extracellularly using a silver chloride electrode inside a glass microelectrode with an approximately 10 μm diameter tip filled with Ringer's solution placed on the surface of the third antennal segment. A reference tungsten electrode was placed inside of the eye or the base of the proboscis. DC signals were acquired and filtered in the same manner as the LFPs.

2.4. Odor stimuli

A continuous air stream of about 300 ml/min was directed at the fly. For odor stimulation, air flow was partially diverted (150 ml/min, 50 % of the total flow) during 0.5 s pulses through a 1 ml syringe with filter paper soaked in 10 μl of an odorant dilution in paraffin oil (Merck, Darmtadt, Germany) and injected again in the main stream using a Stimulus Controller CS 55 (Syntech, Hilversum, The Netherlands) so that final volume was consistently 300 ml/min. To avoid turbulences when joining airstreams, Y-shape connectors were used. Odorants were of the highest grade available (ethyl acetate and pentyl acetate from Merck, Darmtadt, Germany) and were diluted in paraffin oil (vol/vol).

During SSRs, only one sensillum per fly, either ab2 or ab3, was recorded in response to 500 ms pulses of 10^{-2}, 10^{-1} (vol/vol) and pure dilutions of ethyl acetate; and 10^{-2}, 10^{-1} (vol/vol) and pure dilutions of pentyl acetate. The odor stimuli used for LFP and EAG recordings were the same.

2.5. Data analysis

The spikes recorded from contacted sensilla were visualized, stored and first analyzed on a computer using Auto Spike v. 4.0 (Syntech, Hilversum, The Netherlands). When analyzing sensilla containing two ORNs spike sorting –when possible– followed previous protocols (de Bruyne et al., 2001).

Spike counting was performed using the AUTOSPIKE algorithm for detection of spikes and "spike-like" transients in the signal when shape and amplitude are modified during stimulation (see the operation manual of Auto Spike v. 4.0 program for details). Correct application of the time–span settings to recognize spikes can be supervised on the monitor by plotting detected spikes against the original trace.

Spikes were further analyzed using custom routines under Labview 2009 software (National Instruments).

Several parameters of the SSRs were analyzed, including spike frequency, which is presented in the results section of this manuscript as the PSTH of 10 ms bins, and the relative spike amplitude. For spike amplitude analysis, 15 time intervals were defined that corresponded to the 1000 ms spontaneous firing period prior to odor presentation, the 500 ms odor puff, and the after response to the odor over a 10,000 ms recording period (T-0, T-500, T-1100, T-1200, T-1500, T-1800, T-2000, T-2500, T-3000, T-4000, T-5000, T-6000, T-7000, T-8000, T-9000). Although the electrical valve trigger for odorant delivery occurs at T-1000 ms, a response does not initiate until T-1150 ms due to the time required for the stimulus to arrive at the fly. Therefore, the interval starting at T-1200 is the first representation of an actual olfactory response. For each time interval, the first spike was selected and the exact time of the spike upward peak was registered and used in spike amplitude/time graphs. For native sensillum recordings in which spikes from either of two corresponding ORNs appeared first, the first large spike was selected when it could be distinguished from the smaller spike. Spike amplitude was measured as the maximal difference between the upward and downward peaks and was normalized to the amplitude of the spontaneous spike that was selected at the T-0 interval in the same recording. The relative spike amplitude value was then presented as a percentage of the amplitude value of the spontaneous spike.

For each spike, 10 ms recordings were selected beginning 2 ms before the upward peak for the purposes of averaging and shape presentation.

2.6. Statistical analysis

The significance of differences between relative spike amplitude values in response to different odorant stimuli was determined using a repeated-measures ANOVA for which time was considered as an ordinal value. Significant differences were further analyzed for each time point of the response (T-X) by a one-way ANOVA followed by specific comparisons between responses to different odorant concentrations using a Fisher PLSD post hoc test to correct the significance for the comparison of multiple means.

The correlation index r (Pearson) and dispersion graph is presented for each odorant stimulus (odorant type and concentration) in order to thoroughly describe the time course of spike amplitude-frequency relationships and spike amplitude-LFPs. Statistical significance was established by Student's *t*-test.

2.7. Simulation

A 10 ms sample of the average spontaneous spike (beginning 2 ms before the high peak) of the ab2A neuron in the modified sensilla was used as a base for simulation experiments. It corresponds to the spikes selected at T-0 in the SSRs of 10 flies before the first odor stimulus presentation. For the ab2A and ab3A neurons in native sensilla, the average of the corresponding spontaneous large spikes at T-0 was used.

Simulated recordings were obtained by generating trains of these spikes at different rates. Spikes were regularly distributed in time according to spike frequency by using custom routines under Labview 2009 software (National Instruments). For rates that involve spike overlapping, final traces were obtained by the summation of overlapping point values.

3. Results

3.1. Spike amplitudes in ab2A neurons change with odorant stimulation and their time course relates to LFPs

SSRs were obtained for ab2 sensilla to analyze spike frequency and relative amplitude. To describe the kinetics of spike amplitudes in ab2A neurons only, we performed SSR in flies in which the ab2B neurons had been genetically ablated; in heterozygous *Or85a-Gal4/UAS-DTI* flies, selective expression of the diphtheria toxin light chain killed ab2B ORNs expressing Or85a.

Fig. 1 displays the responses of ab2A neurons to ethyl acetate and pentyl acetate. Three concentrations of odorants were tested, beginning at 10^{-2} (vol/vol), because spike amplitude differences are more evident at high odorant concentrations.

Raw SSRs from a single fly (Fig. 1A) are shown as representative of the patterns that were observed in the flies used for analysis (n = 10). A single type of spontaneous spike was detected, as expected in presence of the ab2A neuron alone in the modified ab2 sensillum. In all cases, a reduction in relative spike amplitude was observed directly following a 500 ms pulse of odorant. This reduction persisted even after stimulus cessation. Recovery kinetics depended on both the stimulus type and its concentration. The kinetics of a given response could not be attributed to changes in air pressure within the delivery system, which were negligible (see Fig. S1).

For ethyl acetate, which evokes an excitatory response, high frequency firing was followed by complete inhibition, which increased with increasing concentrations. For pentyl acetate, increasing concentrations diminished spike frequency, following a transient pattern of response that begins to recover prior to stimulus cessation at the highest concentration. However, the pentyl acetate response cannot be considered purely inhibitory because pulses of pure pentyl acetate are able to induce an initial increase in spikes that stops very rapidly. Responses to the 10^{-1} and 10^{-2} concentrations show a graded response as compared to pure pentyl acetate.

Peristimulus-time histograms (PSTH) of 10 ab2A recordings, one per each fly, at 10 ms bin intervals are presented (Fig. 1B), as well as the relative spike amplitude normalized to the size of the spontaneous spike amplitude in each fly at 15 different time intervals, covering all of the studied time period. For each time interval, the first occurring spike is selected, and the exact time of occurrence is used as the X value. Averages of the spike amplitudes and occurrence times of 10 spikes, 1 per fly, were used for the graph. This type of analysis may indicate amplitude progression because variation is minimal under resting conditions and changes gradually in each response interval and during the recovery period. Comparing frequency and amplitude, we see that the maximal amplitude reduction for excitatory responses does not coincide with the maximal spike frequency (that corresponds to T-1200) but occurs later, possibly as a result of it. During the recovery after odor pulses values similar to spontaneous spike frequency before stimulation are achieved long before spontaneous spike amplitude.

Examination of spike amplitude in response to ethyl acetate revealed an initial and fast reduction that is similar at all concentrations and is coincident with the initial fast spike frequency increase. Afterward, the amplitude decreases further at a slower rate that is dependent on odorant concentration, until complete spike abolition is achieved at the highest concentration. The amplitude recovery kinetics are gradual and depend on concentration, with longer recovery times observed at increasing concentrations.

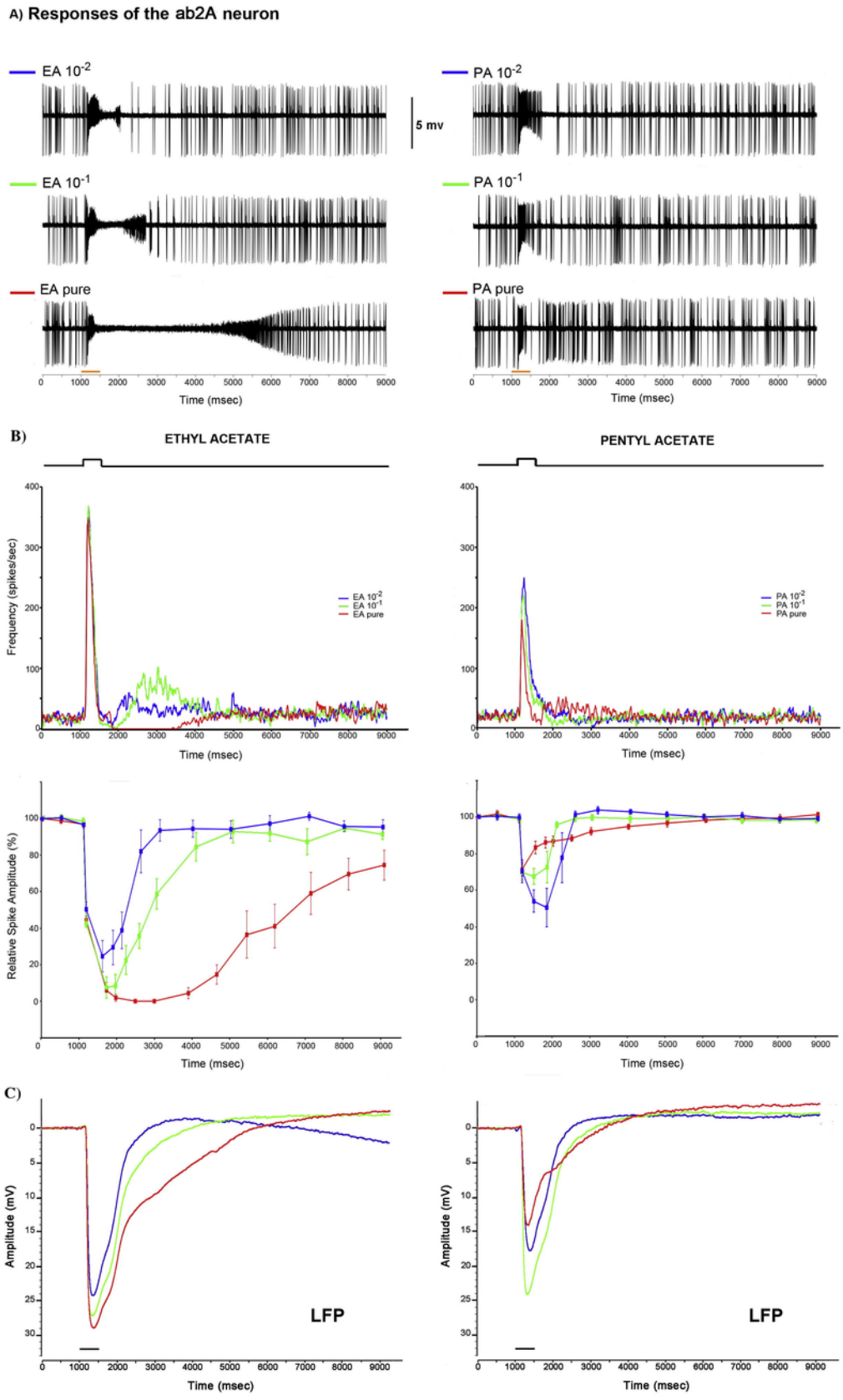


Fig. 1. SSRs of an ab2A neuron in a modified ab2 sensillum lacking the ab2B neuron. A) Original recordings from a single ab2A sensillum in response to three dilutions each of ethyl acetate (EA) and pentyl acetate (PA) in paraffin oil, expressed as vol/vol. The orange line represents a 500 ms odor puff. The colored lines near the odorant and concentration

information serve to identify this information in panels B and C. B) The PSTH of the responses to ethyl acetate (left) and pentyl acetate (right) delivered in 500 ms puffs (upper line). The relative spike amplitudes at different time points compared to the spontaneous spike amplitude at T-0 (lower line). Each point represents an average of 10 spikes (1 per fly) ±SEM. For the X value, the exact time point of the upper peak of each spike is averaged. C) Average LFPs of ab2 sensilla (n = 10,1 per fly) in response to the same stimuli. Note that the 9000 ms time scale is maintained in panels A, B and C. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

In response to pentyl acetate, there is also a fast reduction in spike amplitude, but the posterior slow reduction is negatively correlated with odorant concentration and even initiates recovery before stimulus cessation at the highest concentration. However, recovery speed after stimulus end resembles that for ethyl acetate.

A statistical analysis of the differences between relative spike amplitudes is presented in Table 1. Complete repeated-measures ANOVA of the responses (n = 10 flies) reveals highly significant differences due to time and stimulus as well as interaction between both factors. When the analysis includes differences in time, significant differences begin at T-1200, the first point that reflects a response to the odorant pulse. Although at this time point only differences in spike amplitude between responses to the different odorants become significant (note that in the table only post-hoc comparisons between concentrations of the same odorant are presented). Comparison of particular responses to different concentration pulses showed that in most cases, significant differences corresponded to the responses to ethyl acetate. At T-1500, the spike amplitude of the response to ethyl acetate differed significantly for two of the three possible comparisons At times of maximal inhibition of the response to pure ethyl acetate (T-2500 and T-3000), maximal differences appeared between spike amplitudes in response to the three different odorant concentrations. Differences disappeared gradually at subsequent time points.

For responses to pentyl acetate, significant differences corresponded only to the onset of stimulus presentation or close to it (T-1500, T-1800), where spike amplitude began to recover before the end of the odorant pulse, especially at the highest concentrations. Therefore, the significant differences for pentyl acetate are opposite of those found for ethyl acetate, and spike amplitude is bigger in response to the higher concentration of pentyl acetate than in response to the other concentrations.

We attempted to compare this amplitude reduction with another parameter that follows slow kinetics in response to odorants. The graph displayed in Fig. 1C shows an average of 10 LFPs, one per fly, measured in the ab2 sensilla using the same electrode settings as the SSRs. These measurements were recorded independently of the SSRs to avoid generating similarities by chance. We found that in response to ethyl acetate, which evokes a typical excitatory response, LFP amplitude and recovery kinetics were directly correlated with odor concentration, with the maximal amplitude values and the slowest recovery kinetics being achieved at the highest concentration. Spike amplitude recovery kinetics in response to ethyl acetate resemble these of LFPs.

In response to pentyl acetate, some differences in LFP amplitude were observed depending on concentration but they do not follow a lineal relationship. However, the same transient behavior that was already observed in the SSRs appeared also in response to pure pentyl acetate showing the minimal LFP amplitude. Recovery after response begins before stimulus cessation for this odor concentration, but the second phase of recovery after stimulus cessation resembles the kinetics of the response to ethyl acetate regarding odorant concentration.

Correlation between spike amplitude and frequency at the bin corresponding to each specific time point T-X was statistically analyzed, and dispersion graphs for each odorant and concentration are presented in Fig. 2A. Each graph contains the 15 time points measured for spike amplitude and the regression line. The correlation index r (Pearson) and statistical significance are presented under the corresponding dispersion graph. No significant correlation between spike frequency and amplitude was observed in response to different concentrations of ethyl acetate.

In response to pentyl acetate, some significant correlations between spike frequency and amplitude appeared at pentyl acetate 10^{-1} and pure. However, in these cases spike amplitude changed slightly, most of the points corresponded to complete amplitude recovery and could not effectively describe the variation in the X scale.

Statistical analysis of the correlation between relative spike amplitude and relative amplitude of the averaged LFP at the corresponding time T-X as well as dispersion graphs are presented in Fig. 2B. Note that the relative LFP amplitude was calculated from the average of the normalized LFPs to give the same weight to each fly recording. Normalization for each LFP recording was performed by dividing the amplitude for each point by the amplitude at the time of odor pulse cessation (which in some cases is not the time of the maximal LFP amplitude). Therefore, maximal relative amplitude in LFPs may appear to be lower than -1 mv. Unlike amplitude and frequency of spikes, very significant correlation values were obtained for spike and LFP amplitudes for all tested stimuli and concentrations, with point values covering large amounts of the X axis of the dispersion graphs specially for responses to ethyl acetate.

In summary, relative spike amplitude seems to be statistically correlated to LFP in the ab2A neuron.

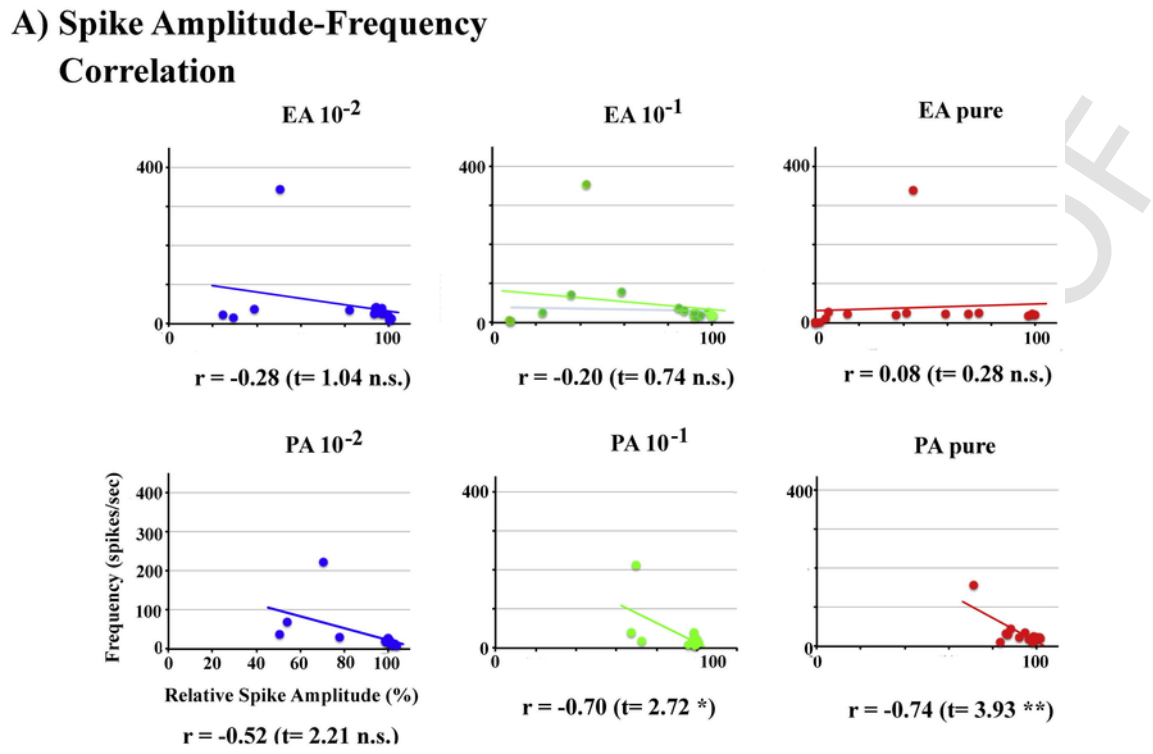
Table 1
A) ANOVA of spike amplitude depending on time and odor stimulus in the modified sensillum ab2, with the ab2A neuron alone. B) ANOVA of spike amplitude at different times (left) and post-hoc comparison of means by the Fisher PLSD (right) with the significant difference set at $P < 0.05$. Differences between spike amplitude responses to two odorant concentrations are identified by both concentrations separated by a dash (the concentration that evokes larger spike amplitude first). Note that only comparisons between concentrations of the same odorant are presented; this is the reason for the lack of detail for T-1200 differences, which appeared only between odorants. (* = $P < 0.05$; ** = $P < 0.01$ and $^{***}P < 0.001$).

Ab2A neuron:

Source	d.f.	MS	F	P
Stimulus (A)	5	5.739	50.94	0.0001***
Subjects w. groups	54	0.113		
Repeated Measure (Time, B)	14	2.602	137.40	0.0001***
Interaction (A × B)	70	0.285	15.04	0.0001***
B × subjects w. groups	756	0.019		

ANOVA of Relative Spike Amplitude differences depending on Time

Time	d.f.	F	P	Ethyl Acetate	Pentyl Acetate
T-500	5, 54	0.348	0.8815 n.s.		
T-1100	5, 54	0.375	0.8637 n.s.		
T-1200	5, 54	11.621	0.0001***		
T-1500	5, 54	32.323	0.0001***	C2-C0, C2-C1	C0-C2
T-1800	5, 54	21.147	0.0001***	C2-C0, C2-C1	C0-C2, C1-C2
T-2000	5, 54	24.897	0.0001***	C1-C0, C2-C0	
T-2500	5, 54	52.474	0.0001***	C1-C0,C2-C0, C2-C1	
T-3000	5, 54	71.140	0.0001***	C1-C0,C2-C0, C2-C1	
T-4000	5, 54	56.782	0.0001***	C1-C0,C2-C0	
T-5000	5, 54	15.609	0.0001***	C1-C0,C2-C0	
T-6000	5, 54	14.744	0.0001***	C1-C0,C2-C0	
T-7000	5, 54	8.247	0.0001***	C1-C0,C2-C0	
T-8000	5, 54	8.311	0.0001***	C1-C0,C2-C0	
T-9000	5, 54	5.826	0.0001***	C1-C0,C2-C0	



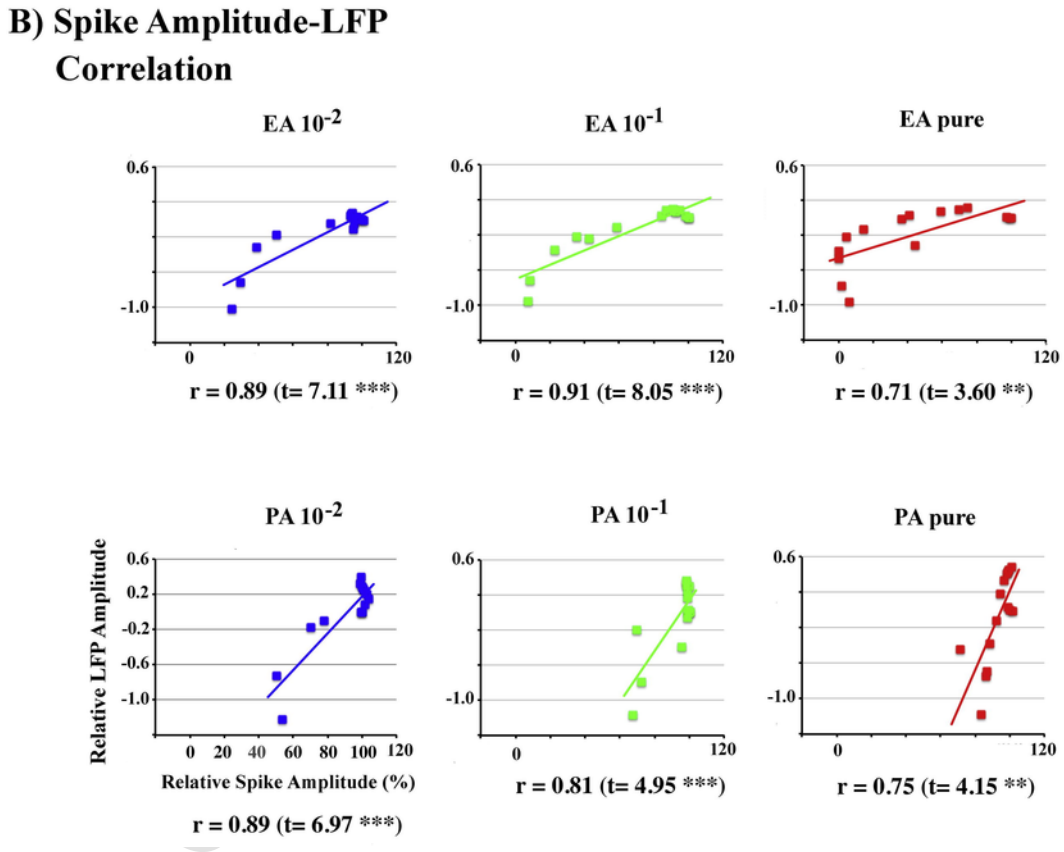


Fig. 2. A) Correlation between spike amplitude and frequency and B) between spike amplitude and LFPs at the bin corresponding to each specific time point T-X and dispersion graphs for each odorant and concentration at the modified sensilla (ab2A alone). Each graph contains the 15 time points measured for spike amplitude. The correlation index r (Pearson) and statistical significance were calculated for n = 15. A lineal regression line is also plotted in each dispersion graph. (* = P < 0.05, ** = P < 0.01, *** = P < 0.001).

To illustrate how spike amplitude and shape evolve during the response to the two tested odorants, we present 10 ms sections comparing spontaneous spikes and those recorded as representatives of different time intervals (Fig. 3) that are aligned at their upper peaks. Each trace represents an average of 10 spikes (one per fly per time period). A sample of the original spike recordings at the same scale at different time points is presented in Fig. S2.

The features of the amplitude time course that are described in Fig. 1B down are directly represented in the spike averages (Fig. 3). During the initial phase of the response to ethyl acetate, which is characterized by fast spike frequency changes (T-1200), direct examination of the single spikes reveals that they do not resemble the original spontaneous spike shape. However, averaging the spikes that are aligned to the upper peak suggests that they represent an intermediate state of the response. After maximal reduction, amplitude recovery is gradual, and for each time interval, the largest amplitude corresponds to the response to the lowest odorant concentration.

In response to pentyl acetate, where spike frequency does not achieve the same maximal values as responses to ethyl acetate, the basic shape of the spontaneous spike is never loose, and a similar reduction degree is observed initially for all odorant concentrations. After that, amplitude recovery starts immediately for the highest odorant concentration and is gradually slower for the other concentrations. This evolution changes its sign after stimulus cessation (T-2000), and complete recovery is finally achieved in the same concentration order as for ethyl acetate, although differences at this phase are very small and no not become statistically significant.

3.2. Reduction of spike amplitude is observed also in native ab2 and ab3 sensilla

To address whether the spike amplitude changes that were observed for ab2A neurons in modified ab2 sensilla could be extended to ORNs in their native environments, we performed SSRs in native ab2 sensilla of wild type flies (Fig. 4).

The original traces shown in Fig. 4A present the contributions of ab2A and ab2B neurons, respectively, corresponding to large and small spikes under resting conditions. Under the conditions of spike abolition used in the previous experiment (i.e., high concentrations of ethyl acetate), a single spike size was observed that likely corresponds to the response of the ab2B neuron that, under resting conditions, shows a relative spike-amplitude of 30% compared to the spontaneous spike of the ab2A neuron.

Fig. 4B compares the spike frequencies and amplitudes that were recorded in the native ab2 sensilla of 11 flies (one sensillum per fly). The spike frequency measurements are presented as a PSTH (top) and include the responses of both neurons because spike amplitude reduction in the ab2A neuron prevented the reliable isolation of the signals of the two neurons during certain moments of the response, especially at the time of stimulus presentation and at spike size reductions of more than 30% of the large spontaneous spike. However, the spike amplitude graphs (Fig. 4B, bottom) represent the response kinetics of the ab2A neuron alone when it could be distinguished from the ab2B response to facilitate comparison with the previous experiment.

Therefore, in this case the minimum relative spike amplitude observed in response to the two highest concentrations of ethyl acetate was 30%, which corresponded to ab2B spikes. Spike amplitudes larger than 30% clearly corresponded to the ab2A neuron, and their reduction levels and kinetics of amplitude recovery matched well with the previous measurements in the modified ab2 sensilla.

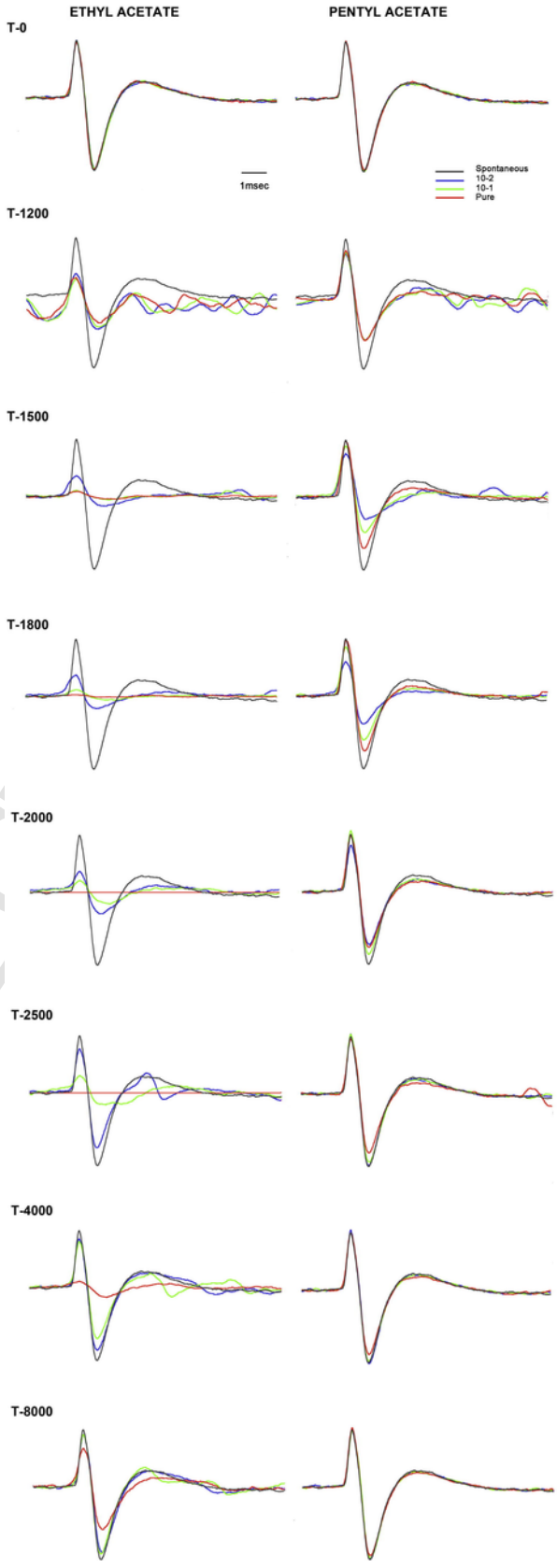


Fig. 3. Average spikes for the values presented in Fig. IB (lower line). Displayed are 10 ms traces at different time points. The gray line represents an average spontaneous spike and is presented for comparison at all time points; the colored lines maintain the same code of stimulus concentrations as that used in Fig. 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

For responses to pentyl acetate, where the range of relative spike amplitude was always above 40% in the ab2A neuron, the spike amplitude kinetics strongly resembled those of previous experiments on ab2A alone. We again found that response recovery initiation was negatively correlated with concentration, with an especially transient response to the highest concentration. However, spike amplitude correlated negatively to odorant concentration as recovery progresses, as previously described.

Statistical analysis of relative spike amplitude in different conditions was performed as in the previous experiment (Table 2). Note that in this case, differences in amplitude are under-represented because we could not record amplitude reductions of greater than 30% of the probable ab2B spikes. Therefore, the range of statistical significance at different times appeared slightly later than in the case of the modified ab2 sensilla, when the size of the ab2A spikes can be distinguished, but the general pattern of differences is quite similar for ethyl acetate, with some additional differences for pentyl acetate.

Correlations between spike amplitude and frequency are presented in Fig. 5A. The same general results found for ab2-modified sensilla also apply to the native ab2 sensilla. A small significant difference was found only for responses to pentyl acetate 10^{-1}. In the rest of the cases no significant correlation was found between spike amplitude and frequency.

With these results, we conclude that the spike amplitude of the ab2A neuron in the native ab2 sensillum obeys the same behavior already described for the ab2A neuron alone. The reduction pattern depends on the odorant and its concentration, and the recovery of spontaneous spike amplitude occurs much later than the recovery of spontaneous spike frequency.

The graph displayed in Fig. 4C shows an average of 10 LFPs, one per fly, measured in the ab2 sensilla using the same electrode settings as the SSRs. In this case, separating the potential contributions of ab2A and ab2B was not possible. We found that in response to ethyl acetate, LFP amplitude and recovery kinetics were directly correlated with odor concentration, with the maximal amplitude values and the slowest recovery kinetics being achieved at the highest concentration as for the modified sensillum. If we consider the contribution of ab2B to the LFP response and the limitations of observing relative spike amplitude reductions under the 30% level, we accept that spike amplitude recovery kinetics in response to ethyl acetate resemble these of LFPs.

In response to pentyl acetate, no differences in LFP amplitudes were observed in relation to concentration, perhaps due to the contribution of the ab2B neuron, but the recovery kinetics after stimulus cessation correlated to odorant concentration, as described above for spike amplitude kinetics.

Statistical analysis of the correlation between relative spike amplitude and relative amplitude of the averaged LFP at the corresponding time T-X as well as dispersion graphs are presented in Fig. 5B. Highly significant correlation values were found in all cases and resembled the results obtained for neuron ab2A alone in the modified sensillum.

In summary, we have established that spike amplitude is statistically correlated to LFP in ab2 sensilla.

The same analysis was performed on the ab3 sensilla, which contains ab3A and ab3B neurons, to determine whether the observations of spike amplitude kinetics for ab2 can be extended to other sensilla (Fig. 6).

In this case excitatory responses were observed in response to both ethyl acetate and pentyl acetate (Fig. 6A). Concerning the isolation of the signal from each neuron in ab3, the size of the small spontaneous spike is approximately 50% that of the large spike; however, with the exception of a region of heavy firing where the typical shape of the spike is lost, both types of spikes could be distinguished by their shapes.

Essentially, the same features described for the ab2 sensilla in response to the excitatory stimulus ethyl acetate were observed for ab3 in response to both stimuli. PSTH, relative spike amplitude (Fig. 6B) and LFP measurements (Fig. 6C) were obtained from recordings in 10 flies (one per fly); as with ab2 measurements, different flies were used for SSRs and LFPs. The recovery of spike amplitude after stimulus cessation in neuron ab3A was markedly delayed compared to the spike frequency kinetics. This delay correlates positively with concentration and appears to be most pronounced in response to pentyl acetate, which also evokes a higher spike frequency than ethyl acetate, as shown in the PSTH in Fig. 6B (top).

A statistical analysis of the differences in spike amplitude is shown in Table 3. It contains the same basic features presented in Table 1 and Table 2. There are significant differences in spike amplitude due to time (T-X) and olfactory stimulus. In this case, because both stimuli are excitatory for the ab3A neuron, differences appear from T-2500 to T-9000 and are always in the same direction, with a larger reduction in spike amplitude in response to higher odorant concentration. Moreover, more differences appear in response to pentyl acetate than in response to ethyl acetate.

Correlations between spike amplitude and frequency are presented in Fig. 7A. The same general results found for previous sensilla apply to the native ab3 sensilla, although the slower recovery of spontaneous spike frequency in this sensillum resulted in some significant correlation for some concentrations of pentyl acetate.

For the LFPs (Fig. 6C), they were not differences in amplitude depending on concentration. However, LFP recovery kinetics slowed as odorant concentration increased in a manner resembling relative spike amplitude kinetics, which was also the case for ab2A neurons.

Correlations between spike amplitude and LFPs (Fig. 7B) were highly significant in almost all cases (except for the response to pure PA) and resembled the results obtained for sensilla ab2.

3.3. Correspondence between sensillum LFP and EAG

Two main types of electrophysiological measurements have been used extensively to describe olfactory function in *Drosophila* at the receptor level: EAGs and SSRs. Spike frequency analysis of SSRs has been the preferred method for studying specific features of particular ORNs. However, in some cases, it has not been possible to detect the contribution of the more general elements of transduction to the olfactory response (for example G-proteins, Yao and Carlson, 2010), even when these contributions have been shown by other methods such as antennal calcium imaging (Ignatious et al., 2014) and are detectable by EAG (Chatterjee et al., 2009).

According to the our experiments, spike frequency analysis of SSRs may not completely describe ORN activity when not completed by an analysis of some parameter reflecting the slow part of the response, either spike amplitude or LFP measured at the single sensillum.

In this experiment, we observed the relationship between these LFPs and EAGs when they were measured as usual at the proximal region of the third antennal segment surface (Fig. 8). This is the region where the large basiconic sensilla ab1, ab2 and ab3 are located.

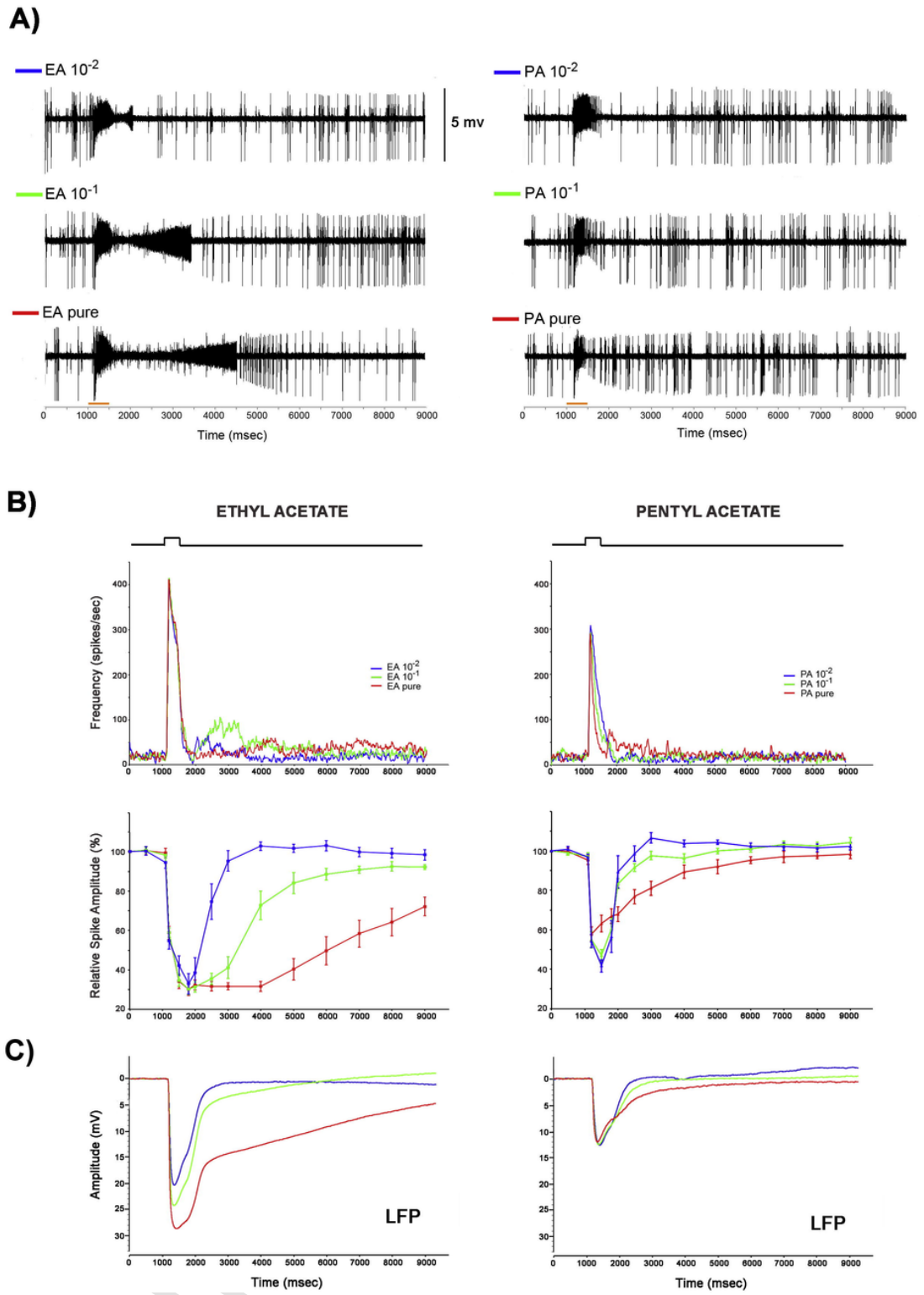


Fig. 4. SSRs of ab2 wild type sensilla. A) Original recordings from a single ab2 sensillum in response to three dilutions each of ethyl acetate (EA) and pentyl acetate (PA) in paraffin oil, expressed as vol/vol. The orange line represents a 500 ms of odor puff. The large spontaneous spikes correspond to the signal of the ab2A ORN, while the small spontaneous spikes correspond to the signal of the ab2B ORN. The colored lines near the odorant and concentration information serve to identify this information in panel B. B) Top, PSTH of the responses of both ab2A and ab2B neurons to ethyl acetate (left) and pentyl acetate (right) delivered in 500 ms puffs (upper line). The relative spike amplitudes at different time points compared to the spontaneous spike amplitude at T-0 (lower line). Each point represents an average of 11 spikes (1 per fly) ±SEM. For the X value, the exact time point of the upper peak of each spike is averaged. C) The average LFPs of ab2 sensilla in response to the same stimuli. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
A) ANOVA of spike amplitude depending on time and odor stimulus in the sensillum ab2. B) ANOVA of spike amplitude at different times (left) and post-hoc comparison of means by the Fisher PLSD (right) with the significant difference set at $P < 0.05$. Differences between spike amplitude responses to two odorant concentrations are identified by both concentrations separated by a dash (the concentration that evokes larger spike amplitude first). Note that only comparisons between concentrations of the same odorant are presented; this is the reason for the lack of detail for T-1500 and T-2000 differences ($^{*} = P < 0.05$; $^{**} = P < 0.01$ and $^{***}P < 0.001$).

Ab2 sensillum:

Source	d.f.	MS	F	P
Stimulus (A)	5	2.771	48.15	0.0001***
Subjects w. groups	60	0.058		
Repeated Measure (Time, B)	14	2.885	164.61	0.0001***
Interaction (A × B)	70	0.185	10.58	0.0001***
B × subjects w. groups	840	0.018		

ANOVA of Relative Spike Amplitude differences depending on Time

Time	d.f.	F	P	Ethyl Acetate	Pentyl Acetate
T-500	5, 60	0.766	0.5780 n.s.		
T-1100	5, 60	0.514	0.7644 n.s.		
T-1200	5, 60	0.347	0.8821 n.s.		
T-1500	5, 60	3.924	0.0038**		
T-1800	5, 60	12.899	0.0001***		C2-C0, C2-C1
T-2000	5, 60	12.135	0.0001***		
T-2500	5, 60	10.072	0.0001***	C2-C0, C2-C1	C2-C1
T-3000	5, 60	31.788	0.0001***	C2-C0, C2-C1	C2-C0, C1-C0
T-4000	5, 60	36.127	0.0001***	C2-C0, C1-C0, C2-C1	C2-C0
T-5000	5, 60	45.309	0.0001***	C2-C0, C1-C0, C2-C1	C2-C0
T-6000	5, 60	29.138	0.0001***	C2-C0, C1-C0, C2-C1	
T-7000	5, 60	25.968	0.0001***	C2-C0, C1-C0, C2-C1	
T-8000	5, 60	19.706	0.0001***	C2-C0, C1-C0	
T-9000	5, 60	19.638	0.0001***	C2-C0, C1-C0, C2-C1	

We plotted the average of the ab2 and ab3 LFPs (Fig. 8A), shown in the previous section for comparison with EAGs recorded in wild type flies in response to the same odorants and concentrations (Fig. 8B). Although we lack the contribution of the ab1 sensilla LFPs in Fig. 8A and the average of the ab2 and ab3 LFPs assumes equal contribution from these two types of sensilla (it has been reported that the contributions of the ab1, ab2 and ab3 sensilla are, respectively, 50%, 30% and 20%; de Bruyne et al., 2001), the final result resembles the corresponding EAGs in Fig. 8B to a reasonable extent. Note that LFP amplitude is higher than EAG amplitude. Because these data are obtained via different recording set-ups, different amounts of current leakage may occur.

Taking together these results suggest that single sensillum LFPs and EAGs reflect the same slow component of the response to olfactory stimuli.

3.4. Can spike overlapping completely explain SSR firing patterns?

As previously reported, excitatory odors can produce intense ORN firing. Here, we show responses to 500 ms odor puffs that are typically excitatory for ethyl acetate and transient for pentyl acetate in ab2A neurons.

At the time of maximum spiking rate, which occurred at approximately T-1200 ms in our recordings (odor response starts at approximately T-1150 ms; see Materials and methods), the typical shape of a spontaneous spike may be lost (Fig. Sup2). It has been shown that ab2 and ab3 sensilla contain around 32 and 44 dendritic branches respectively (Shanbhag et al., 1999), although how many branches corresponded to the A and B neurons have not been determined. Therefore, if spikes generate at dendritic level our observations during intense firing may be caused by spike overlap, which may result in the abnormal spike shapes and amplitude reductions observed.

To test this hypothesis we generated a computer simulation of the patterns produced by spontaneous spike overlapping at different firing rates (Fig. 9A) using the average spontaneous spike of the ab2A ORN from our first experiment on modified ab2 sensilla. In no case did the overlap affect the observed spike frequency. The graph at the top of the figure illustrates the need for rates greater than 1000 spikes/s to reduce the relative amplitude by more than 60% based on overlapping alone, but the maximum firing rate that we observed in the SSRs in response to ethyl acetate was approximately 350 spikes/s, and the relative spike amplitude at the corresponding time (T-1200) was between 40% and 50%. Moreover, for this odor, the maximal amplitude reduction (leaving only between 25% and 0% of the spontaneous spike amplitude) occurs between T-1500 and T-2000, depending on concentration. This means that the maximal amplitude reduction occurs after the end of stimulus exposure and does not relate directly to the firing rate at that moment.

The maximum firing rate that we observed in SSRs in response to pentyl acetate was approximately 190 spikes/s, which corresponds to a relative spike amplitude of 70%. In this case, due to the special transient response observed at the highest concentration, further amplitude reduction was observed in response to lower concentrations of pentyl acetate. Like for ethyl acetate responses, spike overlapping cannot explain relative spike amplitude reduction.

Simulations for ab2A and ab3A in sensilla are presented in Fig. S3. The same general features observed for the modified ab2 sensilla can be applied to the native sensilla. Note that the slopes of the lines in the top graph are steeper than in the simulation using the ab2A neuron in the modified ab2 sensilla. However, this difference cannot be attributed solely to the interaction between the two neurons in a sensillum due to the different genetic backgrounds used.

From these results, it becomes clear that amplitude reduction is not a direct consequence of spike overlapping and is instead related to sensillum status.

Fig. 9B displays original recordings of ab2A responses to 10^{-1} ethyl acetate and pure pentyl acetate that are representative of the two types of studied response: excitatory and transient. At the top, we present a 100 ms firing sequence at the initiation of the response. The successive 20 ms periods represent spontaneous spikes prior to the odor puff, the response initiation, the moment of maximum spiking rate, and intermediate recovery.

The observed spike shapes greatly resemble those generated by direct overlap (Fig. 9A), but there was no correspondence with spiking rate that would be necessary to obtain a similar reduction in spike amplitude. To obtain the actual recording shape and amplitude, an amplitude reduction in the single overlapping spike is also needed.

Fig. 9C displays the average spikes (n = 10) that were recorded in an ab2A neuron at different time intervals (T-0, T-1200, T-1500, T-1800, T-2000, T-2500, T-4000, T-8000) during odor response, starting with a spontaneous spike under resting conditions (T-0). According to shape and amplitude reduction, the initial fast response at T-1200 (in red) seems to befit the odorant but is independent of concentration. However, further spike amplitude reductions (shown in color) are proportional to time and odorant concentration and exhibit opposite patterns for the two odors.

4. Discussion

4.1. Spike amplitudes in ORNs change during odor response

Previous electrophysiological studies of ORN responses to odors in *Drosophila* adults describe activity based on spikes (Clyne et al., 1997; de Bruyne et al., 1999, 2001) and, in a few cases, on slow currents detected in LFPs (Nagel and Wilson, 2011). When describing

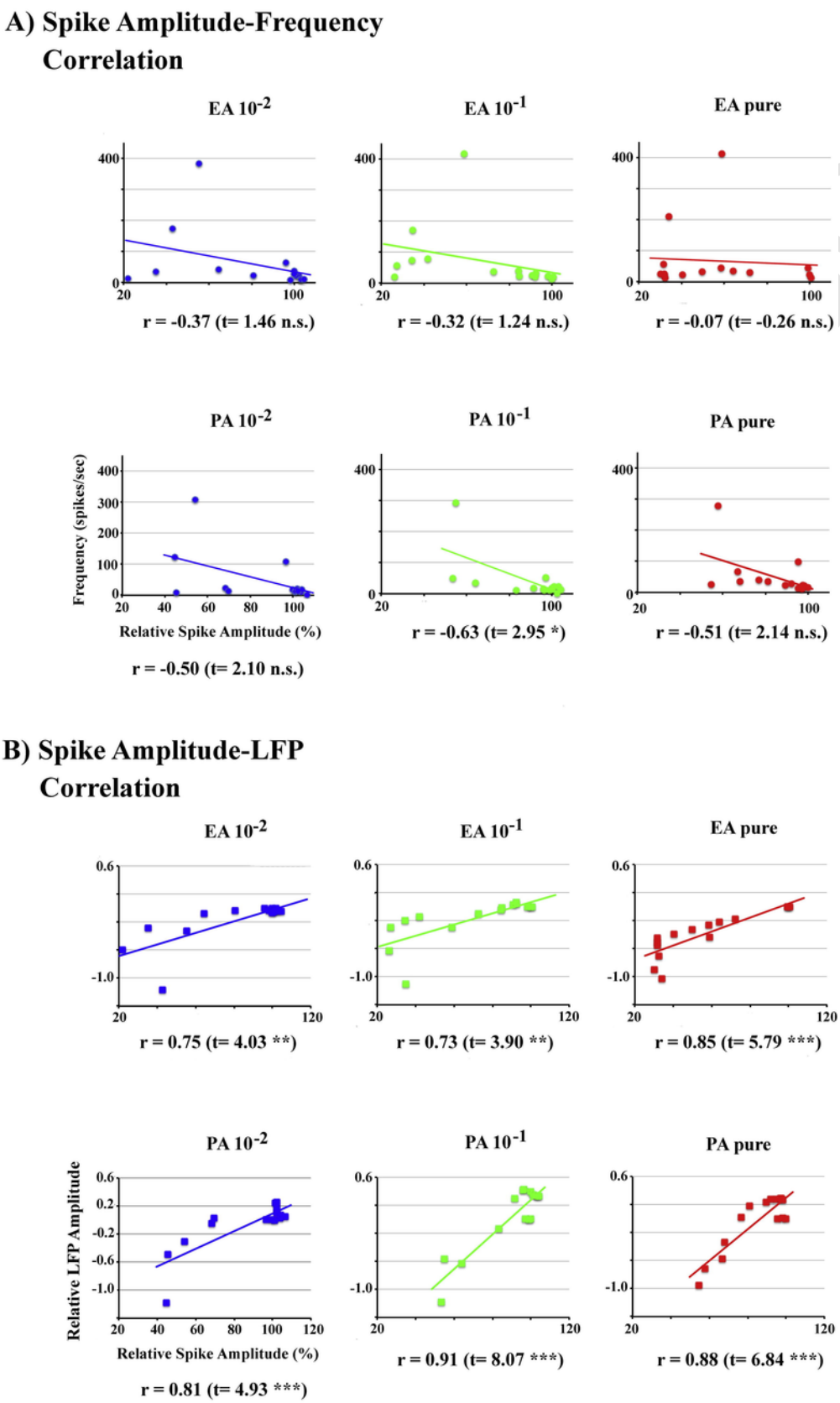

A) Spike Amplitude-Frequency Correlation
EA 10-2
EA 10-1
EA pure
r = -0.37 (t= 1.46 n.s.)
r = -0.32 (t= 1.24 n.s.)
r = -0.07 (t= -0.26 n.s.)
PA 10-2
PA 10-1
PA pure
Frequency (spikes/sec)
Relative Spike Amplitude (%)
r = -0.50 (t= 2.10 n.s.)
r = -0.63 (t= 2.95 *)
r = -0.51 (t= 2.14 n.s.)
B) Spike Amplitude-LFP Correlation
EA 10-2
EA 10-1
EA pure
r = 0.75 (t= 4.03 **)
r = 0.73 (t= 3.90 **)
r = 0.85 (t= 5.79 ***)
PA 10-2
PA 10-1
PA pure
Relative LFP Amplitude
Relative Spike Amplitude (%)
r = 0.81 (t= 4.93 ***)
r = 0.91 (t= 8.07 ***)
r = 0.88 (t= 6.84 ***)

Fig. 5. A) Correlation between spike amplitude and frequency and B) between spike amplitude and LFPs at the bin corresponding to each specific time point T-X and dispersion graphs for each odorant and concentration at the ab2 sensilla. Each graph contains the 15 time points measured for spike amplitude. The correlation index r (Pearson) and statistical significance were calculated for n = 15. A lineal regression line is also plotted in each dispersion graph. (* = P < 0.05, ** = P < 0.01, *** = P < 0.001).

the spiking response, almost all attention has been paid to firing rate. Spike amplitude has only been mentioned in a few studies, which have discussed it as a feature that allows for the separation of the signals of several ORNs within the same sensillum (de Bruyne et al., 2001) and to describe the effects of mutations that reduce the number of olfactory receptors in the membrane (Ha et al., 2014) or the presence of circadian rhythms at the single-unit response level (Krishnan et al., 2008). In all cases, clear spike amplitude differences refer to spontaneous spikes under resting conditions.

In this work, we describe the spike amplitude time course in reference to stimulus response. We show that spike amplitude undergoes a reduction that depends on the ORN, odorant type and odorant concentration but that is not directly correlated with spiking rate. After an initial rapid response, changes occur gradually, and the complete spike inhibition that is achieved in some cases is only an extreme that is preceded by a decreasing spike amplitude. Recovery after maximal reduction also occurs gradually. These features can be observed in both wild-type and genetically modified sensilla that contain only one ORN.

In our experiments, we used medium-to-high odorant concentrations as stimuli (10^{-2}, 10^{-1} and pure, expressed as vol/vol), believing that these conditions would make any effects more apparent.

The olfactory profiles of different ORNs, including those expressing ORs and IRs, have been successfully established based on spike frequency in response to intermediate odor concentrations (10^{-2}) (de Bruyne et al., 1999, 2001; Dobritsa et al., 2003; Hallem et al., 2004; Hallem and Carlson, 2006; Dahanukar et al., 2005; Yao et al., 2005; Van der Goes van Naters and Carlson, 2007; Silbering et al., 2011). We found fewer differences in response recovery kinetics between firing rate and spike amplitude in our data at this concentration because differences increase with increasing concentrations. However, the effects of mutations in other elements that participate in the transduction process and that may affect response kinetics cannot be described by SSR spiking rates with the same efficiency (Yao and Carlson, 2010), although differences caused by mutations have been reported using Ca^{2+} imaging techniques (Ignatious et al., 2014). This may indicate that firing rate analysis does not completely describe ORN activity, especially in the case of responses to high odorant concentrations.

Although gradual changes in spike amplitude in response to odors are commonly observed in *Drosophila* reports that show raw SSR traces (see for example: de Bruyne et al., 2001; Larsson et al., 2004; Abuin et al., 2011; Benton and Dahanukar, 2011), even in response to small odorant concentrations, these changes have traditionally been ignored.

One of the main reasons to disregard spike amplitude differences is the belief that they correspond to artifacts associated with backpropagation of action potentials from the axon where they originate to dendrites, although some reports in other insects suggest that action potentials in sensory sensilla are initiated at dendritic locations (de Kramer, 1985; Hamon et al., 1988; Hamon and Guillet, 1996). Therefore, SSRs as extracellular measurements in the sensillum lymph would record the image of these distorted spikes with changes depending on the distance from their original source.

There are not reports in *Drosophila* on the presence of backpropagation of spikes at the level of olfactory sensilla, perhaps because of the small size of the fly. However, the subject has been thoroughly studied in other insects, especially in moth sensilla in response to pheromones, and even the action potential amplitude has been correlated in some cases with dendrite diameter (Hansson et al., 1994). The electrical properties of the sensillar components have been studied by injecting currents into the sensillum, and equivalent electrical circuits have been designed to understand the effects of backpropagation on the size and shape of spikes (de Kramer et al., 1984; Vermeulen et al., 1996; Rumbo, 1989). However, some contradictory results have been obtained using different experimental conditions, and even direct experiments on olfactory sensillum failed to demonstrate antidromic propagation of action potentials into the dendrite (de Kramer, 1985).

Based on our data, we are in no position to discuss the subject of backpropagation as the cause of the differences we observed. However, some differences in the *Drosophila* SSR measurements in this report compared to backpropagation studies in other insect sensilla should be stated. In our case, we studied direct responses of the sensillum to the natural stimulus, the odor, rather than responses to current injection. In studies of olfactory codes in locust, it has been reported that odorants, but not electrical pulses, evoke reliable, complex spatiotemporal patterns in the antennal lobe (Raman et al., 2010), and perhaps these differences may also apply to coding at the ORN level. In each sensillum, we use the same electrode position to record the responses to different odors and concentrations. Therefore, differences in spike amplitude depending on odorant and concentration are not due to the distance from the spike generator, which is the same in all cases.

On the other hand, spike attenuation associated with passive propagation of spikes has been shown over distances of many microns, and this is not the case in our system. Finally, models that use the cable theory work with ORNs that have a single dendrite (Vermeulen et al., 1996; Gu and Rospars, 2011), but sensilla ab2 and ab3 in *Drosophila* present approximately 32 and 44 dendritic branches, respectively (Shanbhag et al., 1999).

A recent report on signaling of *Drosophila* chemoreceptors studied by patch-clamp recording at the ORN soma (Cao et al., 2016) has shown that under cell-attached recording the firing pattern is similar to that observed for the in vivo SSR preparations. Resemblance includes a reduction in spike amplitude in response to increasing concentrations of an excitatory stimulus. These data support the idea that changes in spike amplitude seen by in vivo SSR are not merely artifacts.

In any case, whether or not the reduction of spike amplitude has functional significance, some consequences should be taken into account for further SSR recordings. From the first SSR recordings in *Drosophila* (de Bruyne et al., 2001), many authors have distinguished the responses of the neurons that share the same sensillum (1–4) by the size of the spike amplitude. Based on this work, it becomes apparent that this classification would not be accurate in response to odors and concentrations that reduce spike amplitude in the A neuron (which produces the large spikes) because small, reduced spikes would be classified as the response of the B neuron.

4.2. Spike amplitude recovery kinetics, LFP and EAGs

In this report, we have shown that spike frequency and spike amplitude follow different kinetics, both during and after responses to odors. The maximal spike frequency is achieved at the beginning of a response, whereas the maximal reduction in spike amplitude is observed at a later time point during the excitatory response, when spike frequency is already reduced. Moreover, spike amplitude recovery

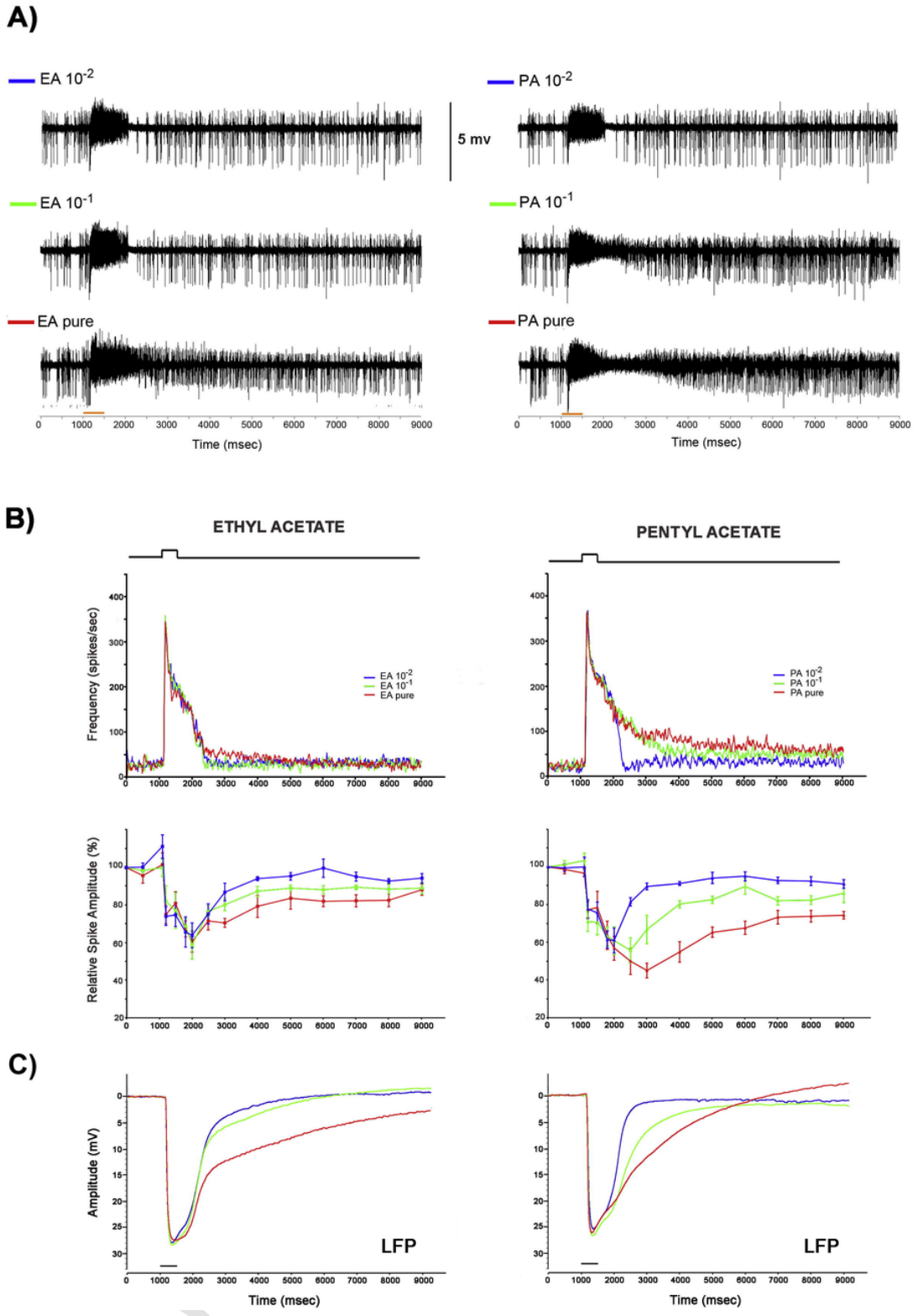


Fig. 6. SSRs of ab3 wild type sensilla. A) Original recordings from a single ab3 sensillum in response to three dilutions each of ethyl acetate (EA) and pentyl acetate (PA) in paraffin oil, expressed as vol/vol. The orange line represents a 500 ms of odor puff. The large spontaneous spikes correspond to the signal of the ab3A ORN, while the small spikes correspond to the signal of the ab3B ORN. The colored lines near the odorant and concentration information serve to identify this information in panel B. B) Top, PSTH of the responses of both ab3A and ab3B neurons to ethyl acetate (left) and pentyl acetate (right) delivered in 500 ms puffs (upper line). Relative spike amplitudes at different time points compared to the spontaneous spike amplitude at T-0 (lower line). Each point represents an average of 10 spikes (1 per fly) ± SEM. For the X value, the exact time point of the upper peak of each

spike is averaged. C) the average LFPs of ab3 sensilla in response to the same stimuli. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
A) ANOVA of spike amplitude depending on time and odor stimulus in the sensillum ab3. B) ANOVA of spike amplitude at different times (left) and post-hoc comparison of means by the Fisher PLSD (right) with the significant difference set at $P < 0.05$. Differences between spike amplitude responses to two odorant concentrations are identified by both concentrations separated by a dash (the concentration that evokes larger spike amplitude first). Note that only comparisons between concentrations of the same odorant are presented. ($^{*} = P < 0.05$; $^{**} = P < 0.01$ and $^{***}P < 0.001$).

Ab3 sensillum:

Source	d.f.	MS	F	P
Stimulus (A)	5	0.581	7.28	0.0001***
Subjects w. groups	54	0.080		
Repeated Measure (Time, B)	14	0.913	61.97	0.0001***
Interaction (A × B)	70	0.041	2.75	0.0001***
B × subjects w. groups	756	0.015		

ANOVA of Relative Spike Amplitude differences depending on Time

Time	d.f.	F	P	Ethyl Acetate	Pentyl Acetate
T-500	5, 54	0.549	0.7384 n.s.		
T-1100	5, 54	0.996	0.4293 n.s.		
T-1200	5, 54	0.428	0.8269 n.s.		
T-1500	5, 54	0.457	0.8066 n.s.		
T-1800	5, 54	0.086	0.9941 n.s.		
T-2000	5, 54	0.149	0.9795 n.s.		
T-2500	5, 54	6.75	0.0001***		C2-C0, C2-C1
T-3000	5, 54	16.915	0.0001***	C2-C0	C2-C0, C1-C0, C2-C1
T-4000	5, 54	15.382	0.0001***	C2-C0	C2-C0, C1-C0, C2-C1
T-5000	5, 54	13.914	0.0001***	C2-C0	C2-C0, C1-C0, C2-C1
T-6000	5, 54	12.594	0.0001***	C2-C0, C2-C1	C2-C0, C2-C1
T-7000	5, 54	10.496	0.0001***	C2-C0	C2-C0, C2-C1
T-8000	5, 54	5.387	0.0004***	C2-C0	C2-C0, C2-C1
T-9000	5, 54	4.562	0.0015**		C2-C0, C1-C0

displayed slow kinetics that are related to the slow currents observed using LFPs and EAGs when activity is measured at the level of the olfactory receptor organ.

EAG recordings in insects to measure antennal response to odors were first performed by Schneider (1957) in experiments with *Bombyx mori* and pheromones and were subsequently used in many other studies (see for example: Steinbrecht and Schneider, 1980; Kaissling and Thorson, 1980; Kaissling, 1971 or the review by Roelofs, 1984). Some authors also tried to compare EAGs to slow DC recordings at the level of a single sensillum (Nagai, 1983a), which we called LFPs. A direct relationship was found between EAG and LFP kinetics, with small differences depending on the position of the antennal EAG recording (Nagai, 1983a,b; Crnjar et al., 1989) and it was suggested that the EAG response is likely to be the summated recording of electrical potentials of many antennal olfactory receptors excited almost simultaneously by odor stimulants (Nagai, 1983a)

In *Drosophila*, the use of EAGs (Venard and Pichon, 1981; Borst, 1984; Alcorta, 1991; Gomez-Diaz et al., 2004, 2006) was sharply reduced after the publication of the first SSRs and the functional mapping of single sensilla in the antenna, with descriptions of the olfactory profiles of their ORNs (de Bruyne et al., 2001).

LFPs have been used in combination with spike frequency to study odor response dynamics in a maxillary palp sensillum ORN model (Nagel and Wilson, 2011). LFP dynamics largely explain the observed odorant responses via a simple model of ligand-receptor interactions, together with an adaptive feedback mechanism that slows the onset of transduction. Spiking dynamics, however, follow a more complex model.

The correlation we found between spike amplitude of the SSRs and LFPs represents for the first time a way to relate these two types of measurements that until now appeared unconnected.

Using computer simulation, we showed that changes in spike amplitude are not caused by the extracellular recording of overlapping spikes and that actual spike amplitude reduction is needed to generate the patterns of spikes observed in single recordings. However, could a change in spike amplitude participate in some of the signaling features that are observed during olfactory reception?

We have observed that spike amplitude reduction and recovery in SSRs are gradual processes in which complete inhibition of spikes is only one extreme of the process. If some small spikes fail to propagate to the synapse, we could have an adaptation scenario, but the point in the periphery at which the action potential becomes too small to propagate is unknown. Adaptation is thoroughly described for ORNs (de Bruyne, 1999; Nagel and Wilson, 2011; Störtkuhl et al., 1999; Liu and Wang, 2001; Ito et al., 2009; Getahun et al., 2012, 2013). Slow recovery of the resting potential may account for adaptation when a new stimulus is presented before the membrane potential has recovered to its resting potential. The same principle could be applied for cross-adaptation in the same ORN: when an ORN is engineered to simultaneously express two different receptors that are activated independently by different ligands, these receptors can cross-adapt (Nagel and Wilson, 2011; Wilson, 2013).

If spike production and amplitude are influenced by the membrane potential and interactions between the electrical fields of ORNs sharing the same sensillum (Van der Goes van Naters, 2013), then previously reported cross-adaptation and inhibition between grouped neurons (Strausfeld and Kaissling, 1986; Vermeulen and Rospars, 2004; Su et al., 2012; Turner and Ray, 2009) could be explained using the same principles.

This report reinforces the idea that complete activity of ORNs should be described by a double measurement, one describing the fast component of the response, such as the spike frequency, and other related to the slow component. Correlation between LFP and spike amplitude suggests that one of those measures could be used for this purpose.

Finally, although a direct relationship between signals measured in the periphery and at synapses in the brain cannot be accepted without additional recordings, could be unified the different signaling features that are observed during olfactory reception in light of our results?

We have observed that spike amplitude correlates significantly with the generator potential measured by the LFPs. If the generator potential is so large that action potentials are not initiated, it is unlikely that there will be any signaling at the release site in the brain. Thus, the interaction between slow currents and spikes through the control of spike amplitude can explain the observation of a complete inhibition of spike production. Could these principles apply also for gradual changes to correlate small spikes and reduced signaling at the synapse?

Further measurements of the consequences of spike amplitude reduction, as measured by SSRs, at the ORN axon close to the first synapse could help with understanding olfactory signaling at the receptor level in more detail.

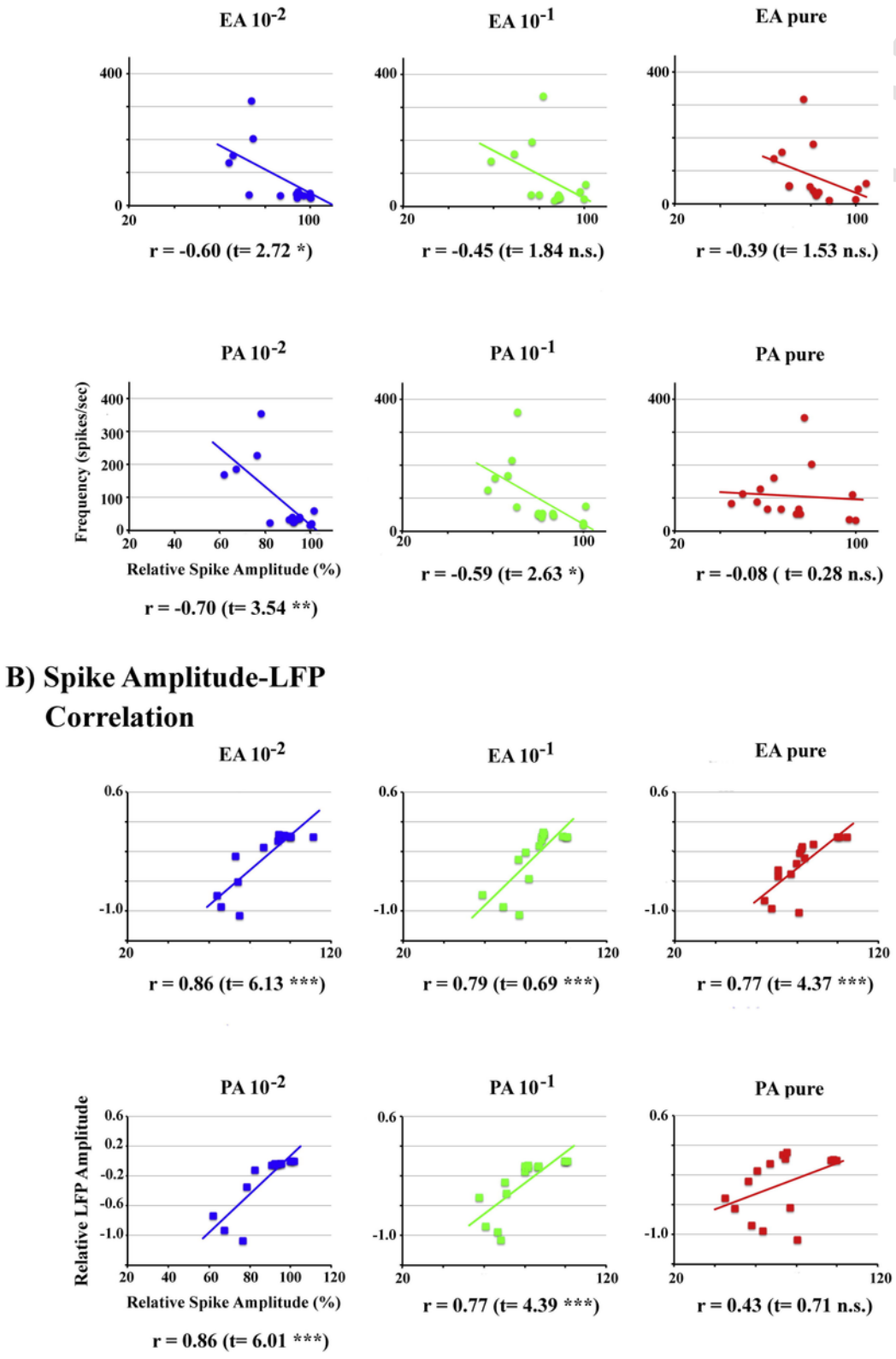

A) Spike Amplitude-Frequency Correlation
EA 10-2
EA 10-1
EA pure
r = -0.60 (t= 2.72 *)
r = -0.45 (t= 1.84 n.s.)
r = -0.39 (t= 1.53 n.s.)
PA 10-2
PA 10-1
PA pure
Frequency (spikes/sec)
Relative Spike Amplitude (%)
r = -0.70 (t= 3.54 **)
r = -0.59 (t= 2.63 *)
r = -0.08 (t= 0.28 n.s.)
B) Spike Amplitude-LFP Correlation
EA 10-2
EA 10-1
EA pure
r = 0.86 (t= 6.13 ***)
r = 0.79 (t= 0.69 ***)
r = 0.77 (t= 4.37 ***)
PA 10-2
PA 10-1
PA pure
Relative LFP Amplitude
Relative Spike Amplitude (%)
r = 0.86 (t= 6.01 ***)
r = 0.77 (t= 4.39 ***)
r = 0.43 (t= 0.71 n.s.)

Fig. 7. A) Correlation between spike amplitude and frequency and B) between spike amplitude and LFPs at the bin corresponding to each specific time point T-X and dispersion graphs for each odorant and concentration at the ab3 sensilla. Each graph contains the 15 time points measured for spike amplitude. The correlation index r (Pearson) and statistical significance were calculated for n = 15. A lineal regression line is also plotted in each dispersion graph. (* = P < 0.05, ** = P < 0.01, *** = P < 0.001).

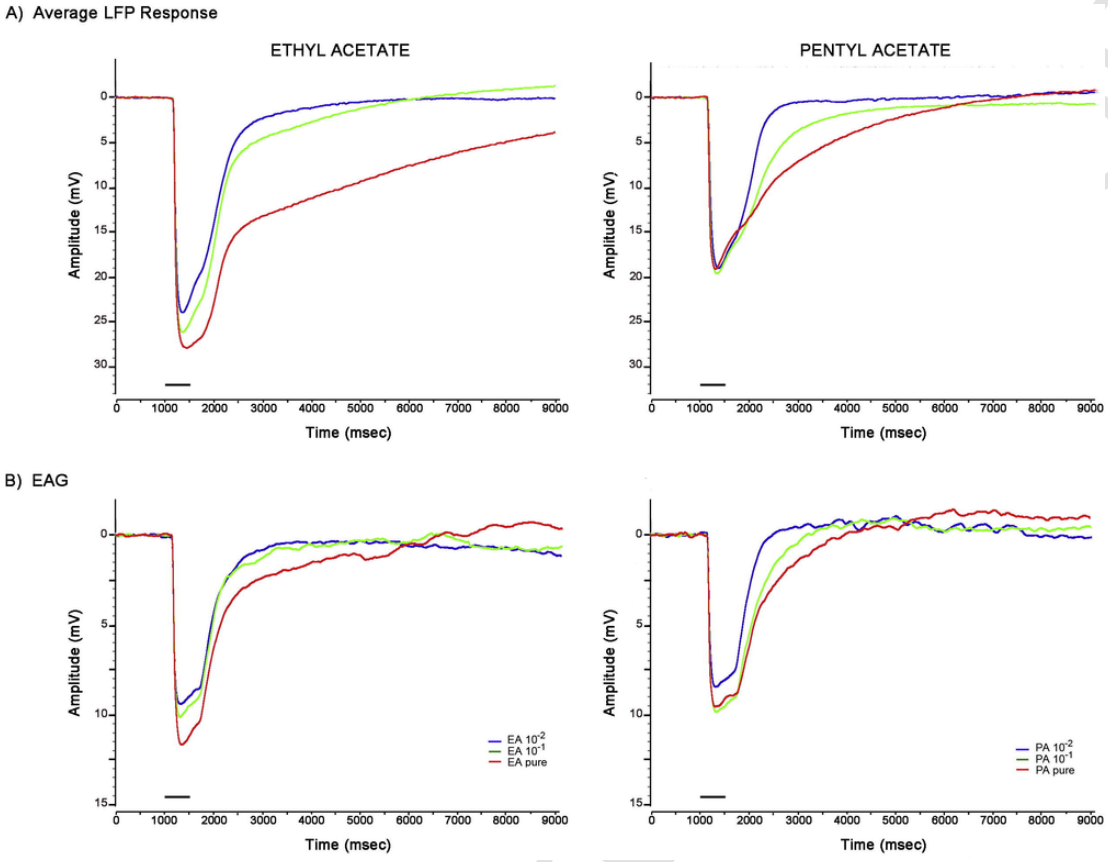


Fig. 8. Comparison between A) the average LFPs of ab2 and ab3 wild type sensilla and B) the EAGs for the same stimuli and concentrations.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jinsphys.2016.09.003.

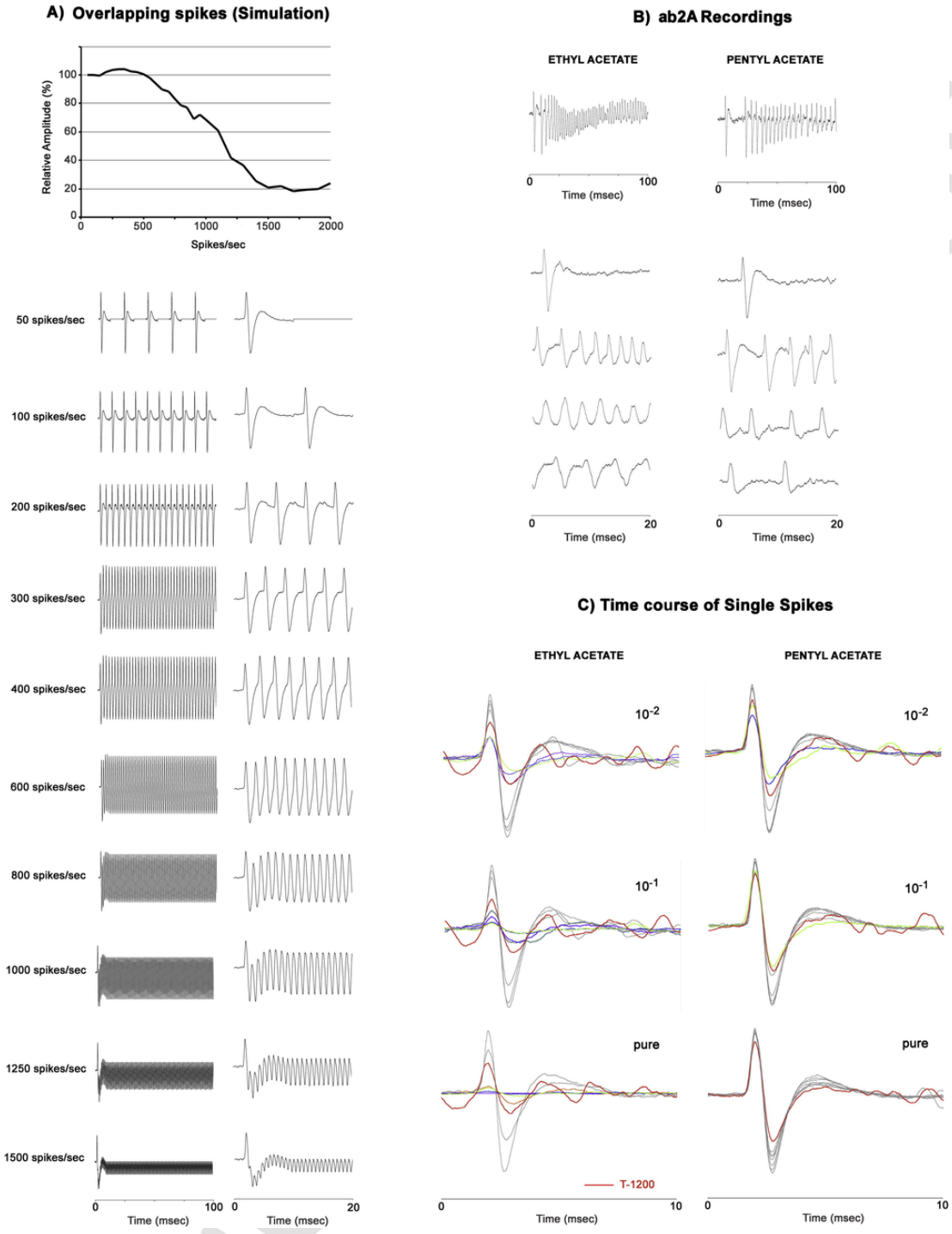

Fig. 9. A) Computer simulation of the effects of spontaneous spike overlapping. Top, a graph showing the dependence of relative amplitude on spike rate. Bottom, the shapes of spikes at different spiking rates in 100 ms (left) and 20 ms (right) samples. B) Original recordings of a single ab2-modified sensillum lacking the ab2B neurons showing 100 ms (top) and 20 ms (bottom) samples for ethyl acetate 10-1 and pure pentyl acetate. The 100 ms samples correspond to the initiation of the response. The 20 ms samples, from top to bottom, correspond to spontaneous spiking, response initiation, maximal firing and intermediate recovery, respectively. C) The 10 ms average spike traces corresponding to different times and concentrations for each odorant. The color red denotes the average spikes at T-1200, the moment of maximal firing rate. When spike amplitude is reduced beyond the level of the red trace, another color is added. Note that the red line exhibits a similar pattern for different concentrations of the same odor but is different between ethyl acetate and pentyl acetate responses. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

References

Abuin, L., Bargeton, B., Ulbrich, M.H., Isacoff, E.Y., Kellenberger, S., Benton, R., 2011. Functional architecture of olfactory ionotropic glutamate receptors. Neuron 69, 44–60. http://dx.doi.org/10.1016/j.neuron.2010.11.042.

Alcorta, E., 1991. Characterization of the electroantennogram in Drosophila melanogaster and its use for identifying olfactory capture and transduction mutants. J. Neurophysiol. 65, 702–714.

Ayer Jr, R.K., Carlson, J., 1992. Olfactory physiology in the Drosophila antenna and maxillary palp: acj6 distinguishes two classes of odorant pathways. J. Neurobiol. 23, 965–982. http://dx.doi.org/10.1002/neu.480230804.

Battefeld, A., Tran, B.T., Gavrilis, J., Cooper, E.C., Kole, M.H., 2014. Heteromeric Kv7.2/7.3 channels differentially regulate action potential initiation and conduction in neocortical myelinated axons. J. Neurosci. 34, 3719–3732. http://dx.doi.org/10.1523/JNEUROSCI.4206-13.2014.

Benton, R., Dahanukar, A., 2011. Electrophysiological Recording from Drosophila Olfactory Sensilla. Cold Spring Harbor Protoc. http://dx.doi.org/10.1101/pdb.prot5630.

Borst, A., 1984. Identification of different chemoreceptors by electroantennogram-recording. J. Insect Physiol. 30, 507–510. http://dx.doi.org/10.1016/0022-1910(84)90032-5.

Cao, L.H., Jing, B.Y., Yang, D., Zeng, X., Shen, Y., Tu, Y., Luo, D.G., 2016. Distinct signaling of Drosophila chemoreceptors in olfactory sensory neurons. Proc. Natl. Acad. Sci. U.S.A. 113, E902–E911. http://dx.doi.org/10.1073/pnas.1518329113.

Chatterjee, A., Roman, G., Hardin, P.E., 2009. Go contributes to olfactory reception in Drosophila melanogaster. BMC Physiol. 9, 22. http://dx.doi.org/10.1186/1472-6793-9-22.

Clyne, P., Grant, A., O'Connell, R., Carlson, J.R., 1997. Odorant response of individual sensilla on the Drosophila antenna. Invert Neurosci. 3, 127–135. http://dx.doi.org/10.1007/BF02480367.

Crnjar, R., Scalera, G., Liscia, A., Angioy, A.-M., Bigiani, A., Pietra, P., Barbarossa, I.T., 1989. Morphology and EAG mapping of the antennal olfactory receptors in Dacus oleae. Entomol. Exp. Appl. 51, 77–85.

Dahanukar, A., Hallem, E.A., Carlson, J.R., 2005. Insect chemoreception. Curr. Opin. Neurobiol. 15, 423–430. http://dx.doi.org/10.1016/j.conb.2005.06.001.

de Bruyne, M., Clyne, P.J., Carlson, J.R., 1999. Odor coding in a model olfactory organ: the Drosophila maxillary palp. J. Neurosci. 19, 4520–4532.

de Bruyne, M., Foster, K., Carlson, J.R., 2001. Odor coding in the Drosophila antenna. Neuron 30, 537–552. http://dx.doi.org/10.1016/S0896-6273(01)00289-6.

de Kramer, J.J., 1985. The electrical circuitry of an olfactory sensillum in Antheraea polyphemus. J. Neurosci. 5, 2484–2493.

de Kramer, J.J., Kaissling, K.E., Keil, T., 1984. Passive electrical properties of insect olfactory sensilla may produce the biphasic shape of spikes. Chem. Senses 8, 289–295.

Dobritsa, A.A., van der Goes van Naters, W., Warr, C.G., Steinbrecht, R.A., Carlson, J.R., 2003. Integrating the molecular and cellular basis of odor coding in the Drosophila antenna. Neuron 37, 827–841. http://dx.doi.org/10.1016/S0896-6273(03)00094-1.

Getahun, M.N., Wicher, D., Hansson, B.S., Olsson, S.B., 2012. Temporal response dynamics of Drosophila olfactory sensory neurons depends on receptor type and response polarity. Front Cell Neurosci. 6, 54. http://dx.doi.org/10.3389/fncel.2012.00054.

Getahun, M.N., Olsson, S.B., Lavista-Llanos, S., Hansson, B.S., Wicher, D., 2013. Insect odorant response sensitivity is tuned by metabotropically autoregulated olfactory receptors. PLoS ONE 8, e58889. http://dx.doi.org/10.1371/journal.pone.0058889.

Gomez-Diaz, C., Martin, F., Alcorta, E., 2004. The cAMP transduction cascade mediates olfactory reception in Drosophila melanogaster. Behav. Genet. 34, 395–406. http://dx.doi.org/10.1023/B:BEGE.0000023645.02710.fe.

Gomez-Diaz, C., Martin, F., Alcorta, E., 2006. The Inositol 1,4,5-triphosphate kinase1 gene affects olfactory reception in Drosophila melanogaster. Behav. Genet. 36, 309–321. http://dx.doi.org/10.1007/s10519-005-9031-x.

Grosjean, Y., Rytz, R., Farine, J.P., Abuin, L., Cortot, J., Jefferis, G.S., Benton, R., 2011. An olfactory receptor for food-derived odours promotes male courtship in Drosophila. Nature 478, 236-40. http://dx.doi.org/ 10.1038/nature10428.

Gu, Y., Rospars, J.P., 2011. Dynamical modeling of the moth pheromone-sensitive olfactory receptor neuron within its sensillar environment. PLoS One. 6:e17422. http://dx.doi.org/ 10.1371/journal.pone.0017422.

Ha, T.S., Xia, R., Zhang, H., Jin, X., Smith, D.P., 2014. Lipid flippase modulates olfactory receptor expression and odorant sensitivity in Drosophila. Proc. Natl. Acad. Sci. U.S.A. 111, 7831–7836. http://dx.doi.org/10.1073/pnas.1401938111.

Hallem, E.A., Carlson, J.R., 2006. Coding of odors by a receptor repertoire. Cell 125, 143–160. http://dx.doi.org/10.1016/j.cell.2006.01.050.

Hallem, E.A., Ho, M.G., Carlson, J.R., 2004. The molecular basis of odor coding in the Drosophila antenna. Cell 117, 965–979. http://dx.doi.org/10.1016/j.cell.2004.05.012.

Hamon, A., Guillet, J.C., 1996. Location and dynamic properties of the spike generator in an insect mechanosensory neuron. J. Comp. Physiol. A. 179, 235–243.

Hamon, A., Guillet, J.C., Callec, J.J., 1988. Initiation and conduction of impulses in mechanosensory neurons: effects of hypoxia. Comp. Biochem. Physiol. 91, 797–805.

Hansson, B., Hallberg, E., Löfstedt, C., Steinbrecht, R., 1994. Correlation between dendrite diameter and action potential amplitude in sex pheromone specific receptor neurons in male Ostrinia nubilalis (Lepidoptera: Pyralidae). Tissue Cell 26, 503–512.

Ignatious Raja, J.S., Katanayeva, N., Katanaev, V.L., Galizia, C.G., 2014. Role of Go/i subgroup of G proteins in olfactory signaling of Drosophila melanogaster. Eur. J. Neurosci. 39, 1245–1255. http://dx.doi.org/10.1111/ejn.12481.

Ito, I., Bazhenov, M., Ong, R.C., Raman, B., Stopfer, M., 2009. Frequency transitions in odor-evoked neural oscillations. Neuron 64, 692–706. http://dx.doi.org/10.1016/j.neuron.2009.10.004.

Kaissling, K.E., 1971. Insect olfaction. In: Beidler, L.M. (Ed.), Handbook of Sensory Physiology, vol. IV/I. Springer-Verlag, Berlin.

Kaissling, K.E., Thorson, J., 1980. Insect olfactory sensilla: structural, chemical and electrical aspects of the functional organization. In: Sattelle, D.B., Hall, L.M., Hildebrand, J.G. (Eds.), Receptors for Neurotransmitters, Hormones and Pheromones in Insects. Elsevier/North Holland Biomedical Press, New York.

Krishnan, P., Chatterjee, A., Tanoue, S., Hardin, P.E., 2008. Spike amplitude of single-unit responses in antennal sensilla is controlled by the Drosophila circadian clock. Curr. Biol. 18, 803–807. http://dx.doi.org/10.1016/j.cub.2008.04.060.

Larsson, M.C., Domingos, A.I., Jones, W.D., Chiappe, M.E., Amrein, H., Vosshall, L.B., 2004. Or83b encodes a broadly expressed odorant receptor essential for Drosophila olfaction. Neuron 43, 703–714.

Liu, Y.H., Wang, X.J., 2001. Spike-frequency adaptation of a generalized leaky integrate-and-fire model neuron. J. Comput. Neurosci. 10, 25–45. http://dx.doi.org/10.1023/A:1008916026143.

Martelli, C., Carlson, J.R., Emonet, T., 2013. Intensity invariant dynamics and odor-specific latencies in olfactory receptor neuron response. J. Neurosci. 33, 6285–6297. http://dx.doi.org/10.1523/JNEUROSCI.0426-12.2013.

Martin, F., Riveron, J., Alcorta, E., 2011. Environmental temperature modulates olfactory reception in Drosophila melanogaster. J. Insect Physiol. 57, 1631–1642. http://dx.doi.org/10.1016/j.jinsphys.2011.08.01.

Nagai, T., 1983. On the relationship between the electroantennogram and simultaneously recorded single sensillum response of the european corn borer, Ostrinia nubilalis. Arch. Insect Biochem. Physiol. 1, 85–91.

Nagai, T., 1983. Spread of local electroantennogram response of the European corn borer, Ostrinia nubilalis. Pestic. Biochem. Physiol. 19, 291–298.

Nagel, K.I., Wilson, R.I., 2011. Biophysical mechanisms underlying olfactory receptor neuron dynamics. Nat. Neurosci. 14, 208–216. http://dx.doi.org/10.1038/nn.2725.

Olsen, S.R., Bhandawat, V., Wilson, R.I., 2010. Divisive normalization in olfactory population codes. Neuron 66, 287–299. http://dx.doi.org/10.1016/j.neuron.2010.04.009.

Raman, B., Joseph, J., Tang, J., Stopfer, M., 2010. Temporally diverse firing patterns in olfactory receptor neurons underlie spatiotemporal neural codes for odors. J. Neurosci. 30, 1994–2006. http://dx.doi.org/10.1523/JNEUROSCI.5639-09.2010.

Riesgo-Escovar, J.R., Woodard, C., Carlson, J.R., 1994. Olfactory physiology in the Drosophila maxillary palp requires the visual system gene rdgB. J. Comp. Physiol. A. 175, 687–693. http://dx.doi.org/10.1007/BF00191841.

Riesgo-Escovar, J.R., Piekos, W.B., Carlson, J.R., 1997. The maxillary palp of Drosophila: ultrastructure and physiology depends on the lozenge gene. J. Comp. Physiol. A. 180, 143–150. http://dx.doi.org/10.1007/s003590050035.

Roelofs, W.L., 1984. Electroantennogram Assays: Rapid and Convenient Screening Procedures for Pheromones. In: Hummel, H.E., Miller, T.A. (Eds.), Techniques in Pheromone Research. Springer-Verlag, Berlin, pp. 131–159.

Rumbo, E.R., 1989. The shape of extracellularly recorded nerve impulses from pheromone receptors. Chem. Senses 14, 361–369.

Sargsyan, V., Getahun, M.N., Llanos, S.L., Olsson, S.B., Hansson, B.S., Wicher, D., 2011. Phosphorylation via PKC regulates the function of the Drosophila odorant co-receptor. Front Cell Neurosci. 16, 5. http://dx.doi.org/10.3389/fncel.2011.00005.

Schlief, M.L., Wilson, R.I., 2007. Olfactory processing and behavior downstream from highly selective receptor neurons. Nat. Neurosci. 10, 623–630. http://dx.doi.org/10.1038/nn1881.

Schneider, D., 1957. Elektrophysiologische Untersuchungen von Chemo-und Mechano-rezeptoren der Antenne des Seidenspinners Bombyx mori L. Z. Vergl. Physiol. 40, 8–41.

Scott, J.W., Sherrill, L., 2008. Effects of odor stimulation on antidromic spikes in olfactory sensory neurons. J. Neurophysiol. 100, 3074–3085. http://dx.doi.org/10.1152/jn.90399.2008.

Shanbhag, S.R., Muller, B., Steinbrecht, R.A., 1999. Atlas of olfactory organs of Drosophila melanogaster 1. Types, external organization, innervation and distribution of olfactory sensilla. Int. J. Insect Morphol. Embryol. 28, 377–397. http://dx.doi.org/10.1016/S0020-7322(99)00039-2.

Shanbhag, S.R., Muller, B., Steinbrecht, R.A., 2000. Atlas of olfactory organs of Drosophila melanogaster 2. Internal organization and cellular architecture of olfactory sensilla. Arthropod. Struct. Dev. 29, 211–229. http://dx.doi.org/10.1016/S1467-8039(00)00028-1.

Silbering, A.F., Rytz, R., Grosjean, Y., Abuin, L., Ramdya, P., Jefferis, G.S., Benton,

R., 2011. Complementary function and integrated wiring of the evolutionarily distinct Drosophila olfactory subsystems. J. Neurosci. 31, 13357–13375. http://dx.doi.org/10.1523/JNEUROSCI.2360-11.2011.

Steinbrecht, R.A., Schneider, D., 1980. Pheromone communication in moth sensory physiology and behavior. In: Locke, M., Smith, D.S. (Eds.), Insect Biology in the Future. Academic Press, New York.

Störtkuhl, K.F., Hovemann, B.T., Carlson, J.R., 1999. Olfactory adaptation depends on the Trp Ca2+ channel in Drosophila. J. Neurosci. 19, 4839–4846.

Strausfeld, C.Z., Kaissling, K.E., 1986. Localized adaptation processes in olfactory sensilla of Saturniid moths. Chem. Senses 11, 499–512. http://dx.doi.org/10.1093/chemse/11.4.499.

Su, C.Y., Menuz, K., Reisert, J., Carlson, J.R., 2012. Non-synaptic inhibition between grouped neurons in an olfactory circuit. Nature 492, 66–71. http://dx.doi.org/10.1038/nature11712.

Turner, S.L., Ray, A., 2009. Modification of CO_2 avoidance behaviour in Drosophila by inhibitory odorants. Nature 461, 277–281. http://dx.doi.org/10.1038/nature08295.

Van der Goes van Naters, W., 2013. Inhibition among olfactory receptor neurons. Front Hum. Neurosci. 7, 690. http://dx.doi.org/10.3389/fnhum.2013.00690.

Van der Goes van Naters, W., Carlson, J.R., 2007. Receptors and neurons for fly odors in Drosophila. Curr. Biol. 17, 606–612. http://dx.doi.org/10.1016/j.cub.2007.02.043.

Venard, R., Pichon, Y., 1981. Etude electro-antennographique de la response peripherique de l'antenne de Drosophila melanogaster a des stimulations odorantes. CR Acad. Sci. Paris 293, 839–842.

Vermeulen, A., Rospars, J.P., 2004. Why are insect olfactory receptor neurons grouped into sensilla? The teachings of a model investigating the effects of the electrical interaction between neurons on the transepithelial potential and the neuronal transmembrane potential. Eur. Biophys. J. 33, 633–643. http://dx.doi.org/10.1007/s00249-004-0405-4.

Vermeulen, A., Rospars, J.P., Lánský, P., Tuckwell, H.C., 1996. Coding of stimulus intensity in an olfactory receptor neuron: role of neuron spatial extent and passive dendritic backpropagation of action potentials. Bull. Math. Biol. 58, 493–512.

Wilson, R.I., 2013. Early olfactory processing in Drosophila: mechanisms and principles. Annu. Rev. Neurosci. 36, 217–241. http://dx.doi.org/10.1146/annurev-neuro-062111-150533.

Yao, C.A., Carlson, J.R., 2010. Role of G-proteins in odor-sensing and CO_2-sensing neurons in Drosophila. J. Neurosci. 30, 4562–4572. http://dx.doi.org/10.1523/jneurosci.6357-09.2010.

Yao, C.A., Ignell, R., Carlson, J.R., 2005. Chemosensory coding by neurons in the coeloconic sensilla of the Drosophila antenna. J. Neurosci. 25, 8359–8367. http://dx.doi.org/10.1523/JNEUROSCI.2432-05.2005.