Graphical abstract

Environmental temperature modulates olfactory reception in *Drosophila melanogaster* **pp xxx–xxx**

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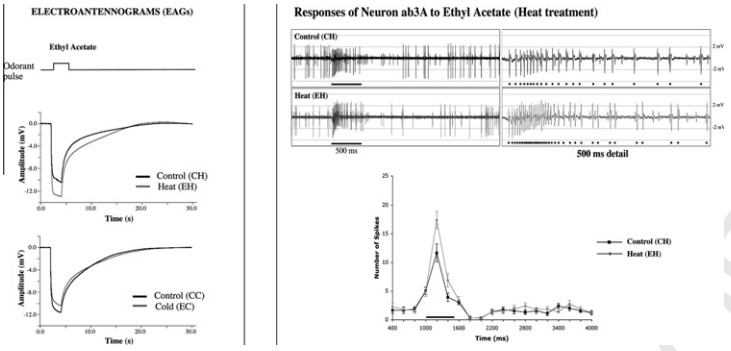

Highlights

▶ *Drosophila melanogaster* adults modify their response to odors depending on temperature. ▶ Changes were observed at the antennal level and in olfactory receptor neurons. ▶ However, temperature treatments do not affect neuron excitability mechanisms. ▶ These signaling changes could initiate previous reported adjusts in perception.

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Environmental temperature modulates olfactory reception in *Drosophila melanogaster*

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ABSTRACT

Sensory systems, including the olfactory system, are able to adapt to changing environmental conditions. In nature, changes in temperature modify the volatility and concentration of odorants in the air. If the olfactory system does not adapt to these changes, it could relay wrong information about the distance to or direction of odor sources. Recent behavioral studies in *Drosophila melanogaster* showed olfactory acclimation to temperature. In this report, we investigated if temperature affects olfaction at the level of the receptors themselves. With this aim, we performed electroantennograms (EAGs) and single sensillum recordings (SSRs) to measure the response to several odorants in flies that had been submitted to temperature treatments. In response to all tested odorants, the amplitude of the EAGs increased in flies that had been exposed to a higher temperature and decreased after cold treatment, revealing that at least part of the reported change in olfactory perception happens at reception level. SSRs of odorant stimulated basiconic sensilla ab2 and ab3 showed some changes in the number of spikes after heat or cold treatment. However, the number and shape of spontaneous action potentials were unaffected, suggesting that the observed changes related specifically to the olfactory function of the neurons.

1. Introduction

In many species, olfaction is the primary sense used to find food, mates and oviposition sites. This sensorial system therefore needs to be able to adapt to environmental changes to produce meaningful information for the animal.

In nature, changes in temperature modify the volatility and concentration of odorants in the air. If the olfactory system does not adapt to these changes, it could relay wrong information about the distance to or direction of odor sources such as food, mating partners or predators. Moreover, the environmental temperature affects many biological phenomena. For example, the ambient temperature determines body temperature in poikilothermic animals inducing changes in the nervous system that affect the behavior of vertebrates (Montgomery and Macdonald, 1990) and influences body size or the length of the developmental period in insects (Ashburner, 1989). For this reason, many animals have developed behavioral and physiological strategies to cope with changes in temperature and other environmental factors (Prosser and Nelson, 1981; Wingfield, 2003). Several behavioral and physiological studies have addressed the ability of the olfactory system to adapt to high odorant concentrations in the environment (Dalton, 2000). In *Drosophila melanogaster*, such adaptation involves changes in both the central (Devaud et al., 2001; Devaud, 2003) and peripheral elements of the olfactory system (Stortkuhl et al., 1999; Deshpande et al., 2000).

Temperature has a profound effect on insects' responses to odors. For example, in the Lepidoptera, temperature modulates the behavioral response to pheromones by reducing male specificity as temperature increases (Linn et al., 1988). The signal amplitude and kinetics of electrophysiological measurements of the antenna (electroantennograms, EAGs) were also modified (Bestmann and Dippold, 1989). Additionally, the number and shape of action potentials in single sensillum recordings (SSRs) in response to pheromones showed temperature-dependent differences (Bestmann and Dippold, 1989; Kodakova, 1996; Kodakova and Kaissling, 1996). These studies demonstrate the contribution of peripheral elements to temperature adaptation in olfaction.

Although there are some studies addressing the influence of temperature in the olfactory discrimination of general odors in turtles (Hanada et al., 1994; Kashiwayanagi et al., 1997), little is known about the temperature-dependent modulation of the response to these odorants in insects. Recent behavioral studies in *D. melanogaster* reported biological acclimation to temperature as to compensate for the change of odorant concentration induced by temperature shifts (Riveron et al., 2009). In that study, the biological effects of temperature were analyzed independently of changes in odorant concentration by studying the persistence of these effects after a period of acclimation.

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In this report, we investigated whether mechanisms of biological adaptation to temperature occur at the level of olfactory reception by using the same experimental approach. EAGs and SSRs were performed on flies that had been submitted to previous heat or cold treatments, and the response to several general odorants was compared to the response of untreated flies. This approach allows measurements at the same temperature in control and experimental individuals and thus concentrates in the search for active adaptation mechanisms. Other temperature-induced changes on passive phenomena such as the fluidity of membrane lipids, the electrical conductance and the kinetics of enzymatic activity, although present in natural conditions, would not be the cause of the observed effects if the time elapsed between temperature treatment and electrophysiological measurement suffices to the flies to acquire the new temperature.

2. Materials and methods

2.1. Fly stocks

Drosophila melanogaster wild-type Canton-S flies were obtained from the Bloomington Stock Center (Bloomington, Indiana, USA). Recordings were restricted to females, considered representative of both sexes. Flies were kept under a 12:12 h light–dark cycle.

2.2. Temperature treatments

Fig. 1 depicts the different temperature treatments applied to the flies before the electrophysiological tests. For the heat treatment experiments, the control (CH) and experimental (EH) groups differed only in the heat period (H). Flies were cultured at 21 °C, and the EH flies were subjected to a 30 °C treatment for 2 days. Individuals were always tested at room temperature (24 °C). For the cold treatment protocol, flies were raised and tested at 24 °C. The experimental group (EC) was subjected to a 15 °C cold period of 48 h (C). In both cases, treatments deviated 9 °C from the initial culture temperature. Flies were tested from 15 to 90 min of being transferred to the recording room.

Control and experimental flies were maintained in a special culture medium containing agarose gel (5 g/l) and sucrose (50 g/l) during the temperature treatment periods. The medium is nearly odorless and therefore did not differentially affect the two groups.

2.3. Electroantennogram recordings (EAGs)

EAGs are extracellular measurements of the voltage changes produced in the antenna in response to odorant stimulation. The recording method, odorant delivery system and data analysis have been described previously (Alcorta, 1991). Briefly, a charcoal-filtered air current produced by an air pump and controlled by a flow meter at 3 ml/s was constantly passed through one of two bottles, either a control bottle containing 5 ml of paraffin oil or a stimulus bottle filled with 5 ml of an odorant diluted in paraffin oil. Odorant pulse generation was controlled with a computer-activated electric valve. Simultaneously, voltage recordings in response to odorant stimulation were amplified and stored by the computer at a 100 Hz sampling rate. With this setup we expected to exclude non-olfactory signals and record only olfactory signals in response to odorant pulses. Moreover, responses to the solvent paraffin oil that is present in the control and stimulus bottle are excluded.

Responses to ethyl acetate, acetone and ethanol (Merck, Germany) diluted in paraffin oil (Merck) were tested. Different flies were recorded for each odorant. In the dose–response curve to ethyl acetate the 0.0001, 0.001 and 0.01 concentrations (expressed as vol/vol in the solvent) were recorded in the same fly. Five pulses of 2 s were recorded, with a period between repetitions that lasted 30 s, except in the 0.1 concentration in the dose–response curve where only one pulse of 2 s was recorded for 50 s to avoid saturation. Each mean trace represents an average of more than 29 flies, with 5 pulses for each fly. Single traces are also presented in some cases to show that the average increased the signal/noise ratio without modifying the original shape of the trace. To evaluate statistically significant differences among the EAGs of control and experimental conditions, the amplitude value was measured for each fly. Amplitude represents maximal voltage deflection after stimulus presentation.

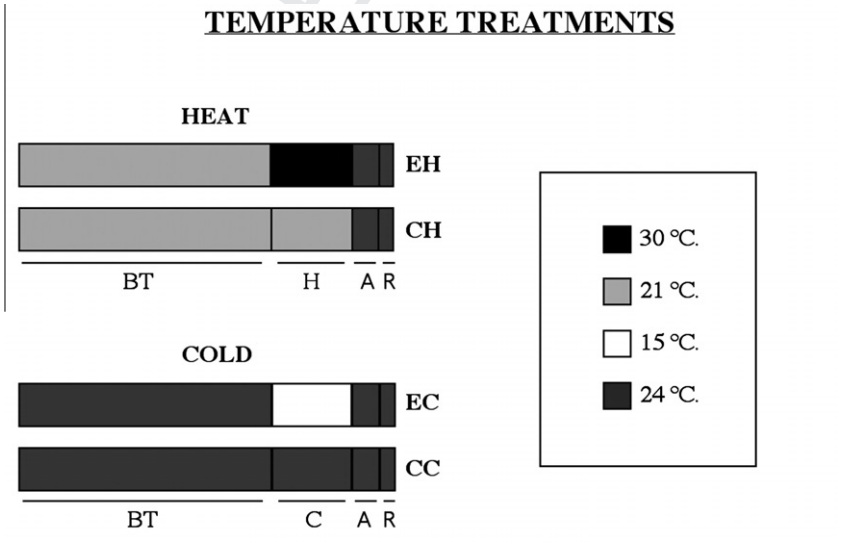

Fig. 1. Temperature treatment protocols. For heat treatment, flies cultured at 21 °C were transferred to 30 °C for 48 h. In the cold treatment protocol, flies reared at 24 °C were moved to 15 °C for the same time period. The acclimation period and the recordings were carried out at 24 °C. BT, breeding temperature; H, heat period; C, cold period; A, acclimation (15–90 min); R, recording (either EAG or SSR). CH, control group and EH, experimental group for the heat treatment. CC, control group and EC, experimental group for the cold treatment.

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2.4. Single sensillum recordings (SSRs)

SSRs were performed on flies mounted in truncated pipette tips. The pipette tip was fixed with wax on a microscope slide, and the antennae were gently placed on a coverslip and stabilized with a glass micropipette (method modified from Clyne et al. (1997)). We used tungsten microelectrodes that were electrolytically sharpened in a KNO_2 solution. The recording electrode was positioned at the sensillum base and the indifferent electrode was placed inside the eye. Electrical signals were recorded using an IDAC 4 amplifier (Syntech, Hilversum, The Netherlands).

We identified the ab2 and ab3 sensilla according to their position, functional profile (de Bruyne et al., 2001) and other specific features (Olsen et al., 2010). The ORN with the largest spike amplitude was termed A, and the ORN with the second-largest spike amplitude was called B.

AC signals were recorded for 10 s and started 1 second before stimulation. A continuous charcoal-filtered humidified air stream of 8 ml/s was directed at the fly. For odor stimulation, air flow was partially deviated (6.6 ml/s) during 0.5 s pulses through a 1 ml syringe containing filter paper soaked in 10 μl of a 0.01 odorant dilution in paraffin oil and then injected into the main stream. Odorants were of the highest grade available (ethyl acetate and acetone from Merck, Germany, and hexanol and ethyl butyrate from Sigma–Aldrich, Germany).

Only one ab2 sensillum per fly was recorded in response to the 4 concentrations of ethyl acetate for the dose–response curves. In the other experiments, either an ab2 or ab3 sensilla per fly were recorded in response to paraffin oil, acetone, hexanol and ethyl butyrate. Action potentials from contacted sensilla were visualized, stored and analyzed on a computer with Auto Spike v. 4.0 (Syntech, Hilversum, The Netherlands) and Labview 2009 software (National Instruments).

Several parameters were analyzed from the recordings: the number of spontaneous spikes before stimulation; the number of spikes during the first 0.5 s after odorant presentation minus spontaneous firing in the previous 0.5 s; and the number of action potentials during the recorded 10 s. To study response kinetics, this last parameter was grouped into 200 ms periods and used to generate a complete histogram of action potentials.

We also measured the amplitudes, maximal voltage differences and shapes of single spontaneous spikes. Average traces for each experimental and control group are presented. When statistical significance was analyzed, a Student's *t*-test was applied for comparing the corresponding parameters in treated and untreated flies.

3. Results

3.1. The environmental temperature influences EAG responses

Canton-S female flies exposed to 48 h of heat or cold treatment (Fig. 1) were tested for their response to odorants using electroantennograms (EAGs). We performed dose-response curves for ethyl acetate for both heat and cold treatments. In order to test if the effects observed in response to ethyl acetate are general or particular for this odorant, we also used other compounds produced in natural conditions by the fermentation of fruits, at concentrations that have been shown to evoque intermediate repellent responses when behavior was tested in a Y-maze (ethyl acetate 0.032, ethanol 0.1 and acetone 0.01). Behavioral responses at these concentrations were affected by the temperature treatments (Riveron et al., 2009).

Statistically significant differences were found in EAG amplitude between flies submitted to the heat treatment (EH) compared to the control flies (CH) for three of the ethyl acetate concentrations (Fig. 2A). The same result is displayed in Fig. 2B that corresponds to the average EAG traces of 30 flies in response to the 0.032 concentration. In all cases, the EAG amplitude in the treated flies was higher than that of the corresponding control animals. Responses were unaffected by the temperature treatment only at the highest concentration, 0.1, far beyond the physiological level. This observation is coincident with the behavioral results (Riveron et al., 2009).

In response to ethanol and acetone the same pattern was found. Response amplitude in the experimental flies (EH) was higher than that of the corresponding control animals (CH). Individual traces are also presented in response to ethanol to show that averaging signals increases the signal/noise ratio without modifying the basic shape of the recordings.

Cold treatment gave the opposite effect; the EAG amplitude of flies submitted to this treatment (EC) diminished significantly compared to the control group (CC), as seen in Fig. 3. In the dose–response curve for ethyl acetate (Fig. 3A) statistically significant differences were found between experimental (EC) and control flies (CC) for all the studied concentrations. The same type of differences appeared in response to ethanol (0.1) and acetone (0.01).

EAG recordings were also measured in another fly stock collected from a natural population. Differences associated with the temperature treatment were also found and resembled the observations in the Canton-S fly stock (data not shown), suggesting that the effect is independent of the fly stock.

The differences in the EAGs that we found between temperature-treated flies and their respective controls point out that the modulation of olfaction by the environmental temperature starts at the olfactory receptor organs. Moreover, as was observed in behavioral tests (Riveron et al., 2009), heat and cold treatments have opposite effects.

3.2. Effects of temperature in particular ORNs

EAGs measure extracellular field potentials in response to odors, reflecting the summated activity of individual ORN responses. To directly address the effect of environmental temperature on the activity of particular ORNs, single sensillum recordings (SSRs) were performed for sensilla ab2 and ab3.

First we tested the dose–response curve of neurons ab2A and ab2B (expressing the odorant receptors 59b and 85a, respectively) for ethyl acetate after heat and cold treatments. Flies underwent the same temperature treatment protocols as for the EAGs.

This odorant has been reported to excite neuron ab2A and ab2B at a high and mild level, respectively (de Bruyne et al., 2001) at intermediate odorant concentrations. Firing in the dose-response curve measure the summation of spikes of both neurons because at odorant concentrations that evoque high spike frequency, spike amplitude is reduced in ab2A making difficult to resolve the response of each neuron.

For each experiment flies from the control and experimental group that are going to be compared statistically have been tested alternately following a block experimental design. Therefore, statistical differences found in an experiment can be related with the previous temperature treatment in the experimental group. However, comparisons among the measurements of different experiments are not allowed because possible differences may be related with other factors of variation among experiments.

Although some significant differences have been found in neuronal firing between flies submitted to temperature treatments and control flies there are not as uniform as the EAGs and depend on neuron and treatment.

In heat-treated individuals, we found a decrease in the number of spikes produced in the ab2 sensilla in response to all the tested concentrations of ethyl acetate (Fig. 4A). However, for cold treated

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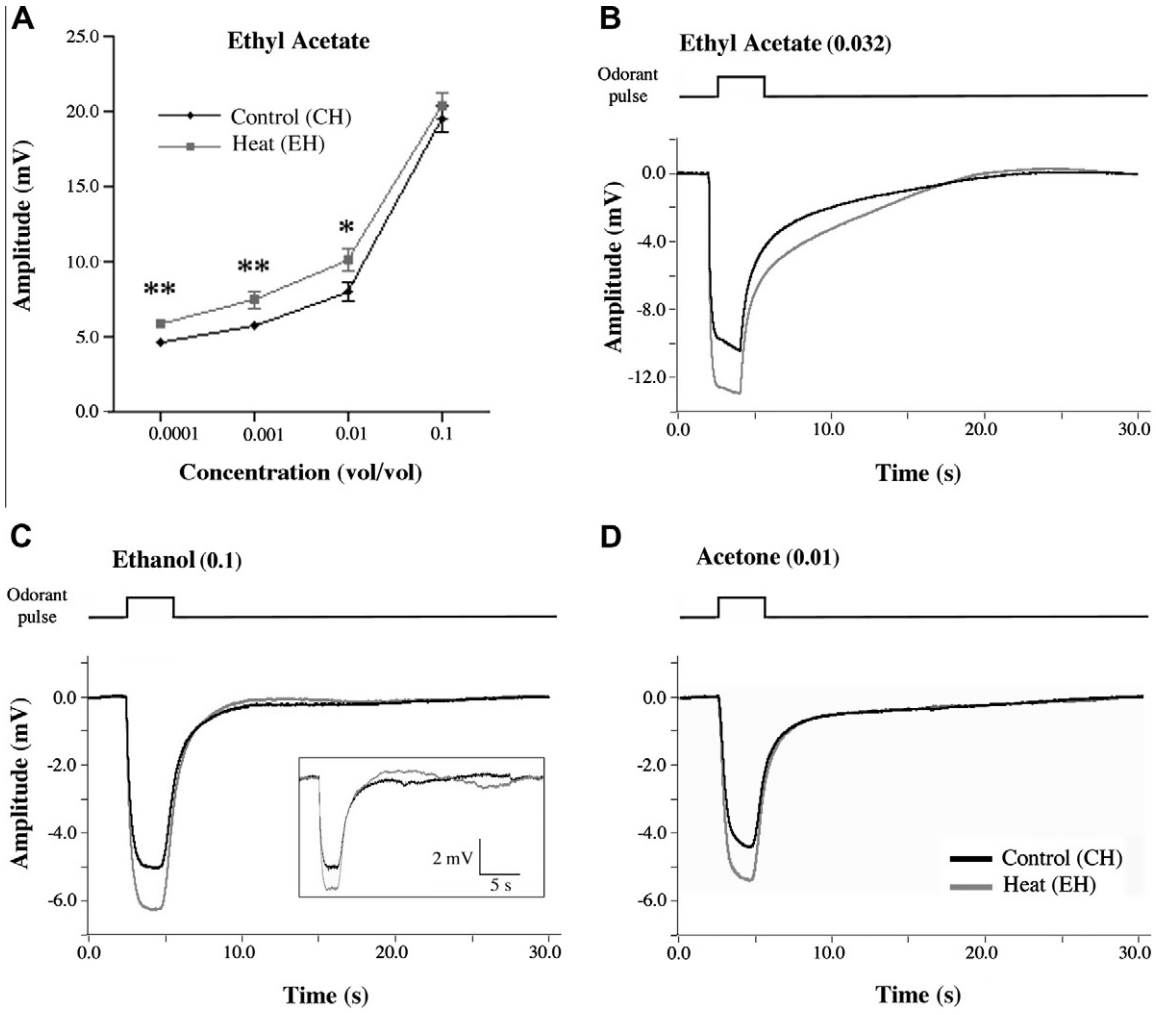

Fig. 2. Electroantennograms (EAGs) of heat-treated flies. (A) Dose–response curve in response to 4 concentrations of ethyl acetate. (B) Mean traces of the EAGs from heat-treated flies (EH) (gray traces) and their controls (CH) (black traces) during 2 s of stimulation with ethyl acetate at the 0.032 concentration (vol/vol). (C) Mean traces of the EAGs from heat-treated flies (EH) (gray traces) and their controls (CH) (black traces) during 2 s of stimulation with ethanol 0.1. Inset: example of EAG traces for individual flies. (D) Mean traces of the EAGs from heat-treated flies (EH) (gray traces) and their controls (CH) (black traces) during 2 s of stimulation with acetone 0.01.

individuals (EC) the response changes significantly only at the 0.0001 concentration compared to the control flies (CC) but in the same direction as for the heat-treated group (Fig. 4B). SSRs shown in Fig. 4 represent the response to ethyl acetate 0.0001 in the heat and cold experiments, respectively.

In order to test if changes in response of ab2 neurons due to temperature treatments are general, new SSRs have been performed in response to other three odorants, acetone, ethyl butyrate and hexanol, all of them at the 0.01 concentration.

Results for the heat treatment experiments are shown in Table 1. Statistically significant changes appeared in response to hexanol. Slight differences in the direction of diminishing response in the experimental group (EH) compared to the controls (CH) appeared in the ab2A neuron, which displayed a small response for this odorant. However, neuron ab2B, that is more responsive to hexanol, showed a significant firing increase.

Responses after cold treatment are shown in Table 2. Significant differences appeared in the response of neuron ab2B to hexanol. However, they are in the direction of diminishing the firing frequency, opposite to the changes with the heat treatment.

SSRs for hexanol after heat and cold treatments highlighting the response of the ab2B neuron are presented in Fig. 5.

Similar experiments have been performed for sensillum ab3 that contains neurons ab3A and ab3B (expressing the odorant receptors 22a and 85b, respectively) in response to four odorants, ethyl acetate, acetone, ethyl butyrate and hexanol, all of them at the 0.01 concentration.

For this sensillum only some differences have been found due to the heat treatment in the ab3A neuron in response to ethyl acetate, in the direction of increasing firing (Table 3, Fig. 6). Cold treatment did not produce any changes (data not shown).

According to this, EAG recordings should correspond to the summation of different types of changes at the ORN level, either increasing or decreasing activity in response to odorants as a consequence of temperature treatment.

These results correspond to measurements that were carried out after all groups had been allowed to come to the same temperature for at least 15 min. Consequently, physical changes that temperature can cause in poikilothermic animals, such as changes in the fluidity of membrane lipids, electrical transmission speed and enzymatic activity at the receptor organs would not account for the changes we saw if this time is sufficient for flies submitted to the temperature treatment to achieve room temperature. Moreover, although membrane composition seems to play an important role in animal acclimation, specially at extreme temperatures, analysis of protein and RNA composition of several *Drosophila* species has shown to be invariant at 15 and 25 °C, in the range of temperatures used for this work (Burr and Hunter, 1969).

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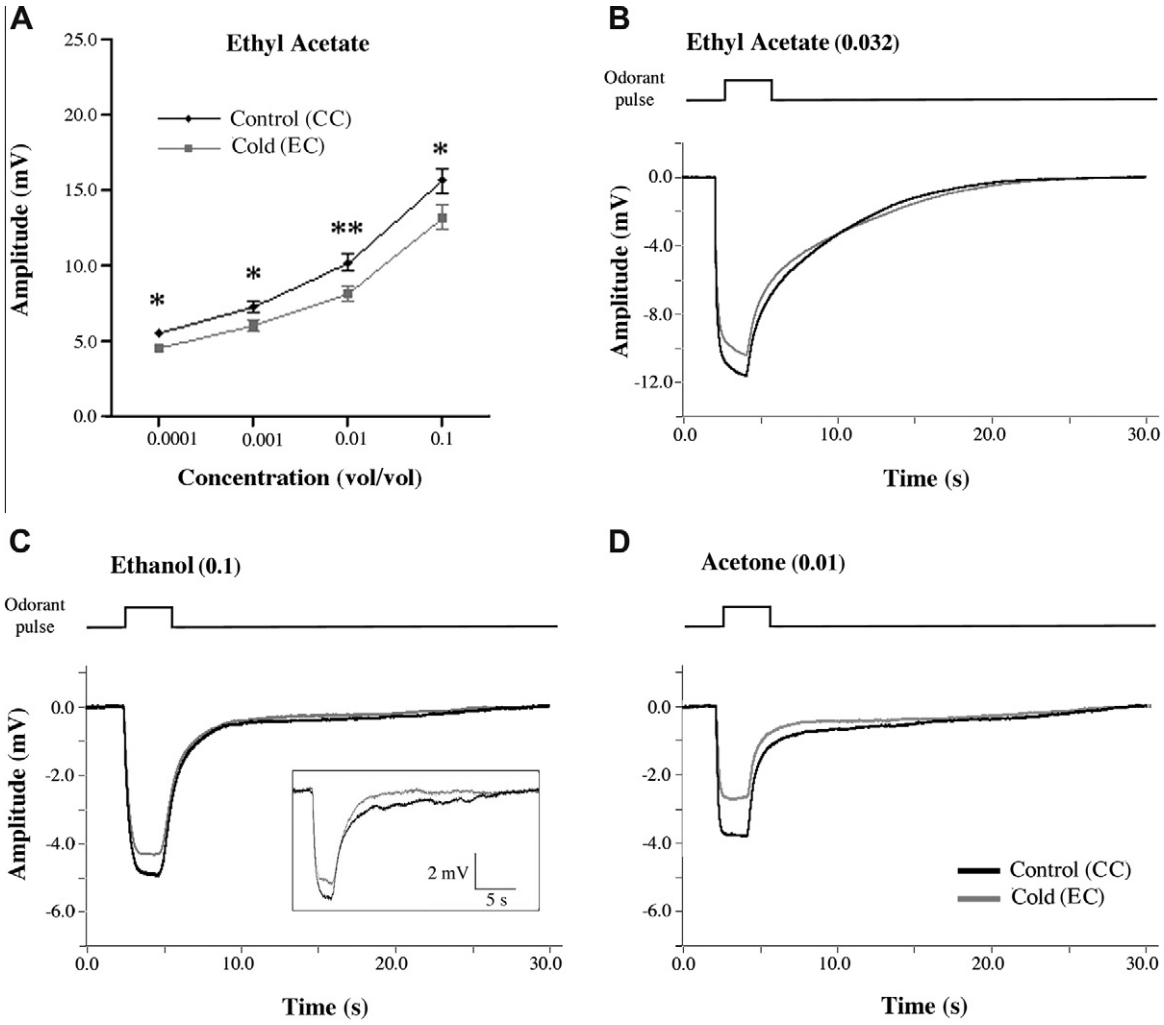

Fig. 3. Electroantennograms (EAGs) of cold-treated flies. (A) Dose–response curve in response to 4 concentrations of ethyl acetate. (B) Mean traces of the EAGs from cold-treated flies (EC) (gray traces) and their controls (CC) (black traces) during 2 s of stimulation with ethyl acetate at the 0.032 concentration (vol/vol). (C) Mean traces of the EAGs from cold-treated flies (EC) (gray traces) and their controls (CC) (black traces) during 2 s of stimulation with ethanol 0.1, Inset: example of EAG traces for individual flies. (D) Mean traces of the EAGs from cold-treated flies (EC) (gray traces) and their controls (CC) (black traces) during 2 s of stimulation with acetone 0.01.

To know if there is a general effect of temperature on ORN excitability that could be related to membrane composition changes, we analyzed the frequency, amplitude and shape of the spontaneous spikes of treated and untreated flies.

The frequency of spontaneous action potentials was not affected in the ab2 or ab3 sensilla (data not shown).

Table 4 describes the amplitudes of the spontaneous spikes showing that neither the heat nor the cold treatments produced any significant difference in amplitude for the four ORNs analyzed.

Fig. 7 displays the spontaneous spikes of ORNs ab2A and ab3A from heat-pretreated (EH) and untreated (CH) flies. The average traces for each class are depicted in the inset at the right of each graph. Fig. 8 illustrates the data for the cold treatment experiment. The temperature treatments produced no significant differences in the spike shape. These data collectively suggest that temperature does not modulate neuron excitability mechanisms and instead mediates specific changes in the response to odors.

4. Discussion

Our electrophysiological analyses of EAGs and SSRs show that environmental temperature modulates the response to general odors at the receptor level as well as at particular ORNs. Such an effect has already been seen in the olfactory perception of flies submitted to the same temperature treatments as we used (Riveron et al., 2009). It is therefore possible that behavioral adaptation could initiate at the peripheral part of the olfactory system.

Environmental temperature affects two processes: the concentration of volatiles in the air and the internal physiology of the animal. In poikilothermic animals, body temperature, membrane fluidity (Wodtke, 1981; Kashiwayanagi et al., 1997; Ohtsu et al., 1998; Overgaard et al., 2005) and metabolic rate (Gillooly et al., 2001; Clarke, 2006) are particularly affected. In our experiments, we tried to clarify if active mechanisms also participate in the olfactory acclimation to temperatures within the normal range for *D. melanogaster* that was seen at the behavioral level in response to general odors (Riveron et al., 2009). With this aim, flies were treated with temperature protocols that either increased or decreased the environmental temperature by 9 °C and studied between 15 and 90 min after treatment ending. Although the electrophysiological recordings of treated and control animals were performed at the same temperature and odorant concentration, we were still able to find persistent effects of acclimation to the previous temperature at the olfactory receptor level even with such small temperature shift. In fact, olfactory reception appears to be affected by the thermal history; the fly responded differently depending on the environmental temperature to which it has been

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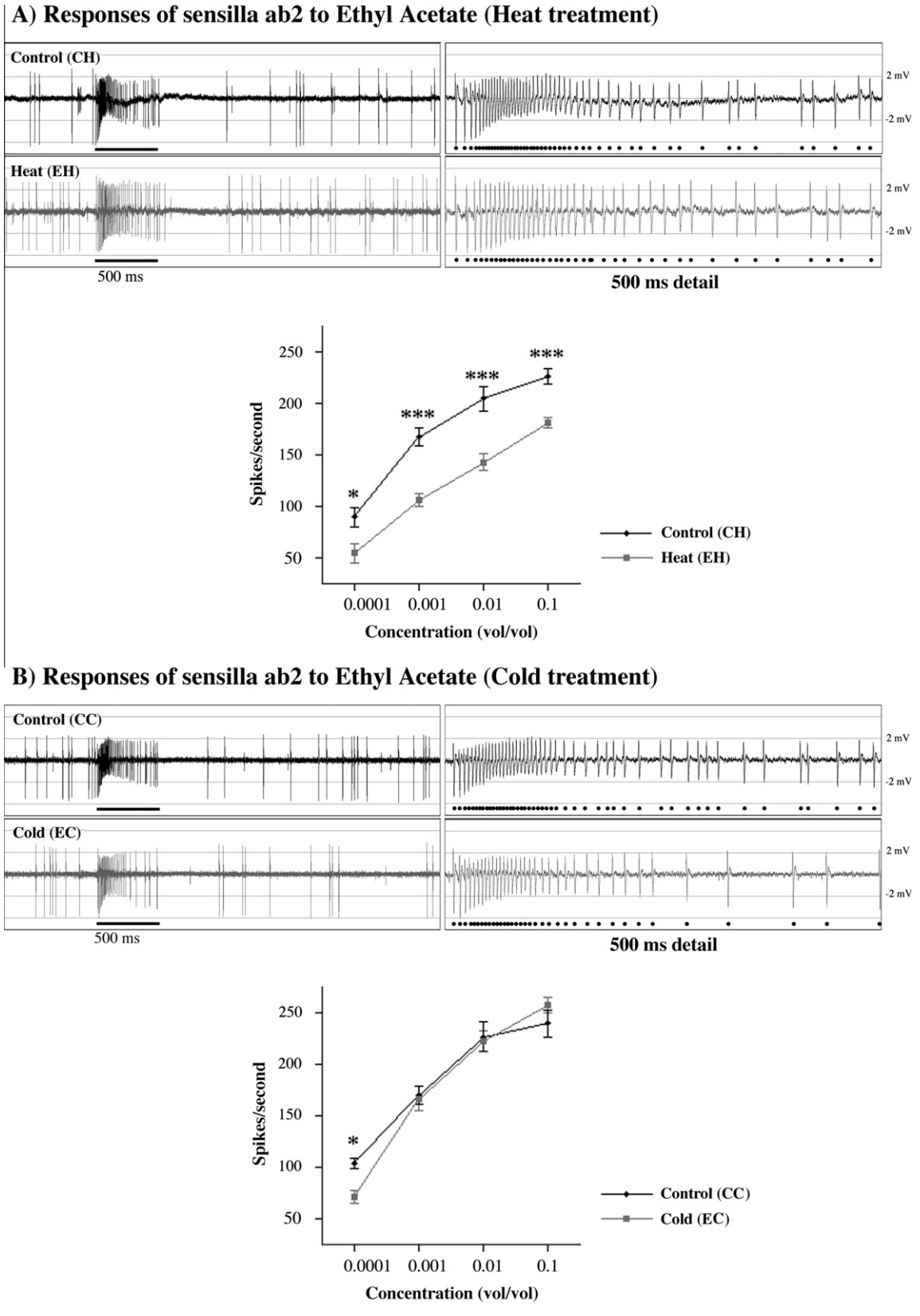

Fig. 4. Single sensillum recordings (SSRs) of the ab2 sensillum to ethyl acetate. (A) Responses of ab2 sensilla in heat-treated animals. Top left: examples of individual recordings from sensillum ab2 to ethyl acetate 0.0001. Top right: details of the individual recordings, each spike is marked with a point. Bottom: dose–response curve in response to 4 concentrations of ethyl acetate. (B) Responses of ab2 sensilla in heat-treated animals. Top left: examples of individual recordings from sensillum ab2 to ethyl acetate 0.0001. Top right: details of the individual recordings, spikes are marked with a point. Bottom: dose–response curve in response to 4 concentrations of ethyl acetate. $^{*}P < 0.05$. $^{***}P < 0.001$.

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Table 1
ab2 single sensilla recordings from heat treated flies and their controls.

Neuron type	Control flies (CH)		Heat treated flies (EH)		*t* Value	*P*
	n	Means ± SE (spikes/s)	*n*	Means ± SE (spikes/s)		
Acetone 10^{-2}						
Neuron A (Or59b)	10	71.20 ± 5.74	10	70.20 ± 4.98	−0.13	0.897
Neuron B (Or85a)		30.80 ± 3.68		33.20 ± 3.57	0.47	0.645
Ethyl Butirate 10^{-2}						
Neuron A (Or59b)	10	109.80 ± 2.59	10	105.60 ± 9.24	−0.44	0.667
Neuron B (Or85a)		62.60 ± 4.96		74.80 ± 13.02	0.88	0.393
Hexanol 10^{-2}						
Neuron A (Or59b)	10	39.00 ± 8.32	10	14.60 ± 7.10	−2.23	0.039*
Neuron B (Or85a)		100.40 ± 5.32		135.00 ± 10.27	2.99	0.008**

Spike frequency during the half second of stimulation minus the number of spontaneous spikes in the previous half second ± Standard Error. Values for each neuron type for the 3 odors used are shown. *n* = number of flies. Control and heat treated flies were compared with a *t*-student test. *P* = probability.
* $P < 0.05$.
** $P < 0.01$.

Table 2
ab2 single sensilla recordings from cold treated flies and their controls.

Neuron type	Control flies (CC)		Heat treated flies (EC)		*t* Value	*P*
	n	Means ± SE (spikes/s)	*n*	Means ± SE (spikes/s)		
Acetone 10^{-2}						
Neuron A (Or59b)	10	91.78 ± 17.09	10	92.80 ± 17.09	0.036	0.971
Neuron B (Or85a)		55.33 ± 21.45		25.2 ± 4.1	−1.45	0.165
Ethyl butirate 10^{-2}						
Neuron A (Or59b)	10	78.22 ± 8.74	10	59.2 ± 7.29	−1.68	0.111
Neuron B (Or85a)		64.00 ± 8.08		71.00 ± 5.60	0.72	0.479
Hexanol 10^{-2}						
Neuron A (Or59b)	10	35.33 ± 6.11	10	38.20 ± 4.55	0.38	0.707
Neuron B (Or85a)		91.56 ± 5.92		65.40 ± 6.98	−2.82	0.0117*

Spike frequency during the half second of stimulation minus the number of spontaneous spikes in the previous half second ± Standard Error. Values for each neuron type for the 3 odors used are shown. *n* = number of flies. Control and cold treated flies were compared with a *t*-student test. *P* = probability.
* $P < 0.05$.

previously subjected. Similar findings were reported in electrophysiological studies of Lepidoptera responses to pheromones at different temperatures (Bestmann and Dippold, 1989). In that case, the hysteresis phenomenon (different values depending on previous temperature treatments) was observed in EAG amplitude as well as in the number of action potentials, even with previous temperature treatments as short as a few seconds or minutes. If changes mediated by temperature acclimation remain for some time in the ORNs, signal facilitation or inhibition by previous higher or lower temperatures could be understood.

In our case, flies subjected to a heat period had an increased EAG amplitude, whereas flies subjected to cold had a decreased EAG response for all tested odorants. However, SSR changes did not follow such homogeneous pattern. Therefore, although the EAG involves the summation of extracellular voltage changes in response to odorants in the antenna such changes are not equal in all the ORNs. We see that the same temperature treatment had diverse effects in different neurons (for example, the effects of heat in the response of ORNs ab2 and ab3A to ethyl acetate decreasing or increasing spike number, respectively), that the two treatments had opposite effect in the same neuron (increasing spike number in ab2B in response to hexanol in heat treated animals and decreasing it in cold treated ones) but also heat and cold treatments may have the same effect in the same sensilla (diminishing the spike number in response to ethyl acetate in ab2). This last behavior corresponds to ORNs that showed their maximum activity at an intermediate temperature and was already described in neurons that respond to pheromones in ~~Antheraea pernyi~~ (Bestmann and Dippold, 1989).

On the other hand, only the response to some odorants change significantly for those ORNs whose activity was affected by the temperature treatment. It has been reported that the same ORN responds differentially depending on the odorant and even producing activation and inhibition in some cases (de Bruyne et al., 2001; Schuckel et al., 2009). If different cell components mediate the olfactory response to distinct odors, non-uniform alteration of these components by temperature may have diverse effects. In our case, taking into account that the flies were tested between 15 and 90 min after the temperature treatment, passive changes in lipid fluidity of membranes or enzymatic activity due to temperature cannot explain the changes in the EAGs and SSRs. Indications of some active and specific adaptation phenomena can be also concluded because spontaneous action potentials were unaffected by the temperature treatments.

Because the effects of environmental temperature shifts on olfactory perception have been previously studied in *Drosophila* following the same protocol (Riveron et al., 2009), we can consider whether acclimation at both the perception and reception levels follows a consistent pattern. In EAG recordings, a linear relationship between the odor concentration and the amplitude has been described (Alcorta, 1991). Thus, in our experiments, the effect of the heat treatment is the same as the response to a greater odor concentration: the amplitude increases. Similarly, the effect on the cold-treated animals is the same as the response to a less concentrated odorant: the amplitude is smaller. However, in the olfactory behavior study, animals subjected to heat treatment were less sensitive to the odorant than the controls at the linear part of the dose response curve (repellent response region); they behaved like they were subjected to a less concentrated odorant. In the cold treatment, the opposite occurred; they behaved as though they had been subjected to a more concentrated odorant. The apparent

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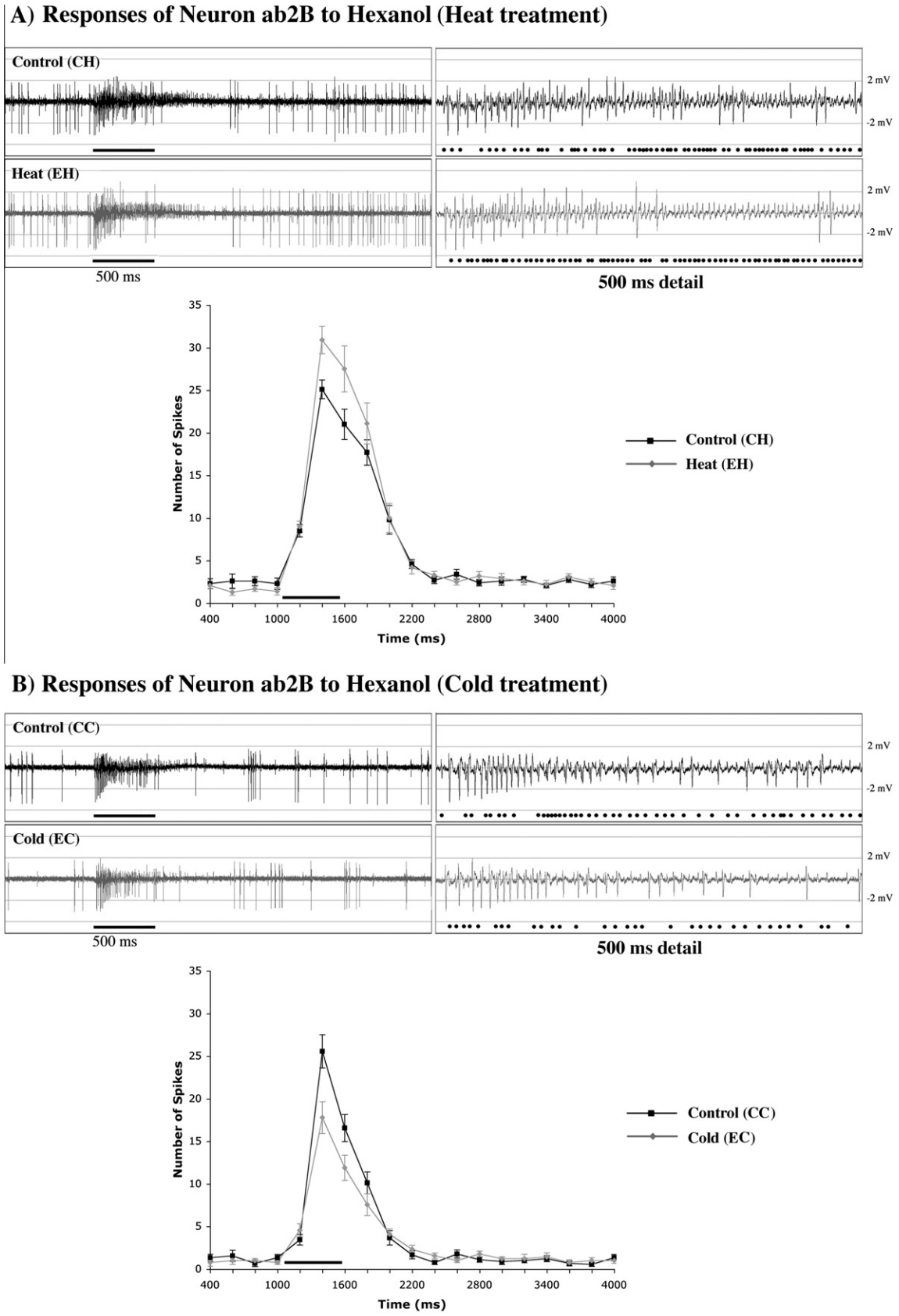

Fig. 5. Single sensillum recordings (SSRs) of ab2B neurons to hexanol 0.01. (A) Responses of ab2B neurons in heat-treated animals. Top left: examples of individual recordings from sensillum ab2. Top right: details of the individual recordings, spikes from ab2B neuron are marked with a point. Bottom: Mean spike frequency recorded in ab2B neurons at 200 ms intervals. (B) Responses of ab2B neurons in cold-treated animals. Top left: examples of individual recordings from sensillum ab2. Top right: details of the individual recordings, spikes from ab2B neuron are marked with a point. Bottom: Mean spike frequency recorded in ab2B neurons at 200 ms intervals.

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Table 3
ab3 single sensilla recordings from heat treated flies and their controls.

Neuron type	Control flies (CH)		Heat treated flies (EH)		*t* Value	*P*
	n	Means ± SE (spikes/s)	*n*	Means ± SE (spikes/s)		
Ethyl acetate 10^{-2}						
Neuron A (Or22a)	11	38.54 ± 3.34	8	57.00 ± 5.22	3.12	0.006**
Neuron B (Or85b)		34.18 ± 4.02		30.26 ± 3.66	−0.69	0.497
Acetone 10^{-2}						
Neuron A (Or22a)	11	86.5 ± 9.48	8	71.10 ± 7.12	1.33	0.202
Neuron B (Or85b)		37.82 ± 9.28		24.76 ± 5.12	−1.11	0.283
Ethyl Butirate 10^{-2}						
Neuron A (Or22a)	11	132.36 ± 16.62	8	137.00 ± 16.52	0.19	0.850
Neuron B (Or85b)		78.18 ± 20.10		62.50 ± 28.36	−0.47	0.648
Hexanol 10^{-2}						
Neuron A (Or22a)	11	60.92 ± 5.42	8	69.00 ± 5.10	1.05	0.309
Neuron B (Or85b)		152.54 ± 10.96		144.50 ± 7.72	−0.56	0.586

Spike frequency during the half second of stimulation minus the number of spontaneous spikes in the previous half second ± Standard Error. Values for each neuron type for the 4 odors used are shown. *n* = number of flies. Control and heat treated flies were compared with a *t*-student test. *P* = probability.

** $P < 0.01$.

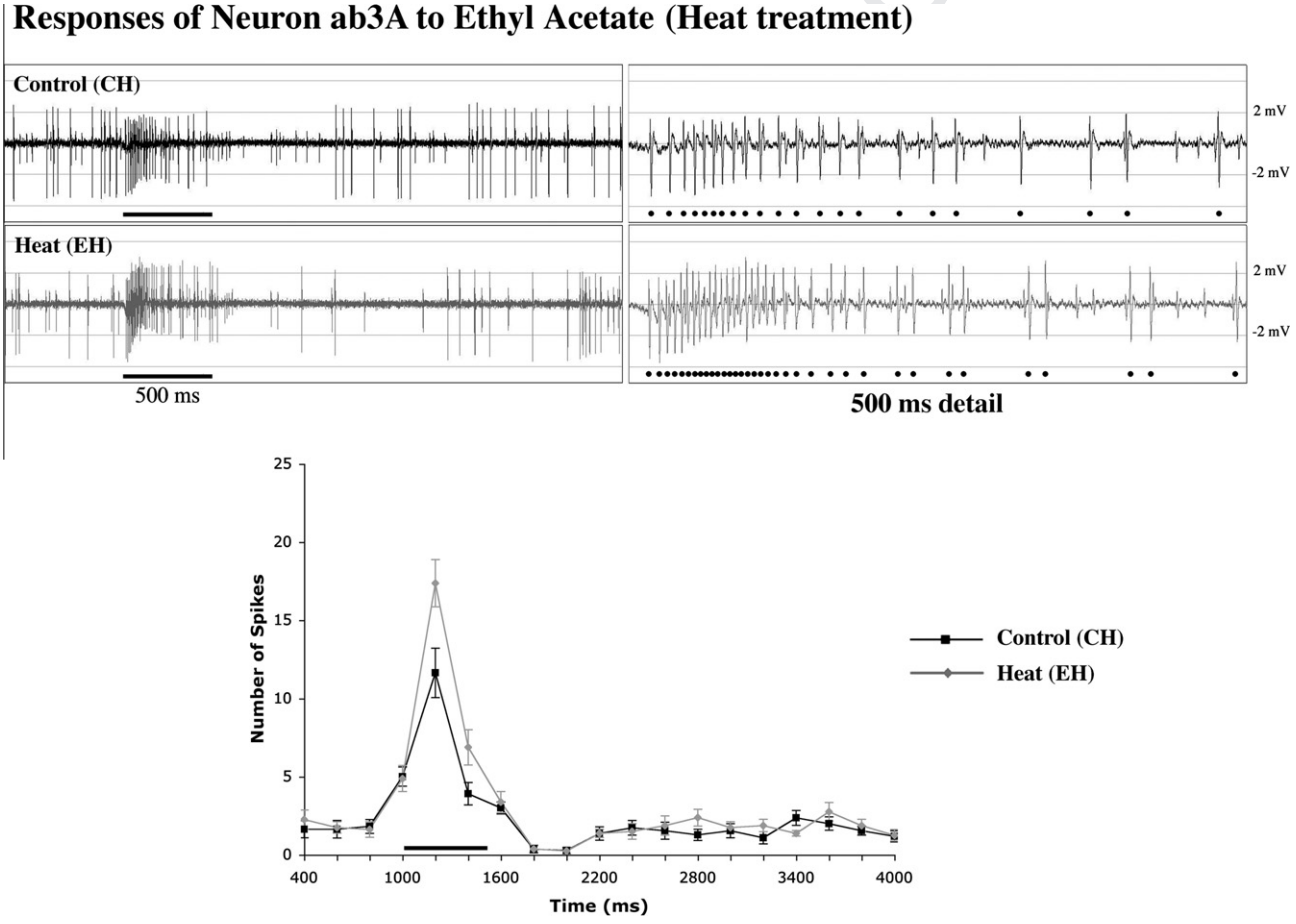

Fig. 6. Single sensillum recordings (SSRs) of ab3A neurons from heat-treated animals to ethyl acetate 0.01. Top left: examples of individual recordings from sensillum ab3. Top right: details of the individual recordings, spikes from ab3A neuron are marked with a point. Bottom: Mean spike frequency recorded in ab3A neurons at 200 ms intervals.

discrepancy between both sets of data – receptor electrophysiology and behavior – disappears if we consider the individual SSR results and the current knowledge of the olfactory coding of general odors in *Drosophila*. The observed variability in the effects of temperature in sensilla ab2 and ab3 precludes simple and global interpretations because the effects of temperature treatments in the EAGs correspond to the summation of heterogeneous changes at the ORN level.

If we think in the response to each odorant, it has been shown to be combinatorial (Hallem and Carlson, 2006). Any odorant cue will produce the activation and/or inhibition of several ORN classes, each expressing one different olfactory receptor, whose input will be interpreted in the brain. The odor intensity would be codified not only for the strength of activation of individual neurons, but also for the combination of activity across the ORN classes (Hallem and Carlson, 2006). This receptor activity pattern produces

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Table 4
Amplitude of spontaneous spikes.

Neuron type	Control flies (CH)		Heat treated flies (EH)		*t* Value	*P*
	n	Mean ± SE (mV)	*n*	Mean ± SE (mV)		
ab2A	10	4.92 ± 0.35	11	4.48 ± 0.35	−0.90	0.380 ns
ab2B	9	1.42 ± 0.10	11	1.51 ± 0.10	0.61	0.547 ns
ab3A	11	3.99 ± 0.26	8	4.13 ± 0.25	0.39	0.702 ns
ab3B	11	1.58 ± 0.12	8	1.51 ± 0.11	−0.43	0.672 ns
	Control flies (CC)		Cold treated flies (EC)			
ab2A	10	4.36 ± 0.31	7	4.54 ± 0.34	0.38	0.707 ns
ab2B	9	1.33 ± 0.09	7	1.41 ± 0.09	0.60	0.556 ns
ab3A	8	3.89 ± 0.36	8	3.66 ± 0.24	−0.53	0.603 ns
ab3B	8	1.61 ± 0.22	8	1.67 ± 0.11	0.25	0.808 ns

Amplitude of spontaneous spikes in the four neurons analyzed for heat-treated flies and their controls (top) and cold-treated flies and their controls (bottom). *n* = number of flies. Mean amplitudes of control and temperature treated flies were compared using the Student's *t*-test. *P* = probability, ns = not significant.

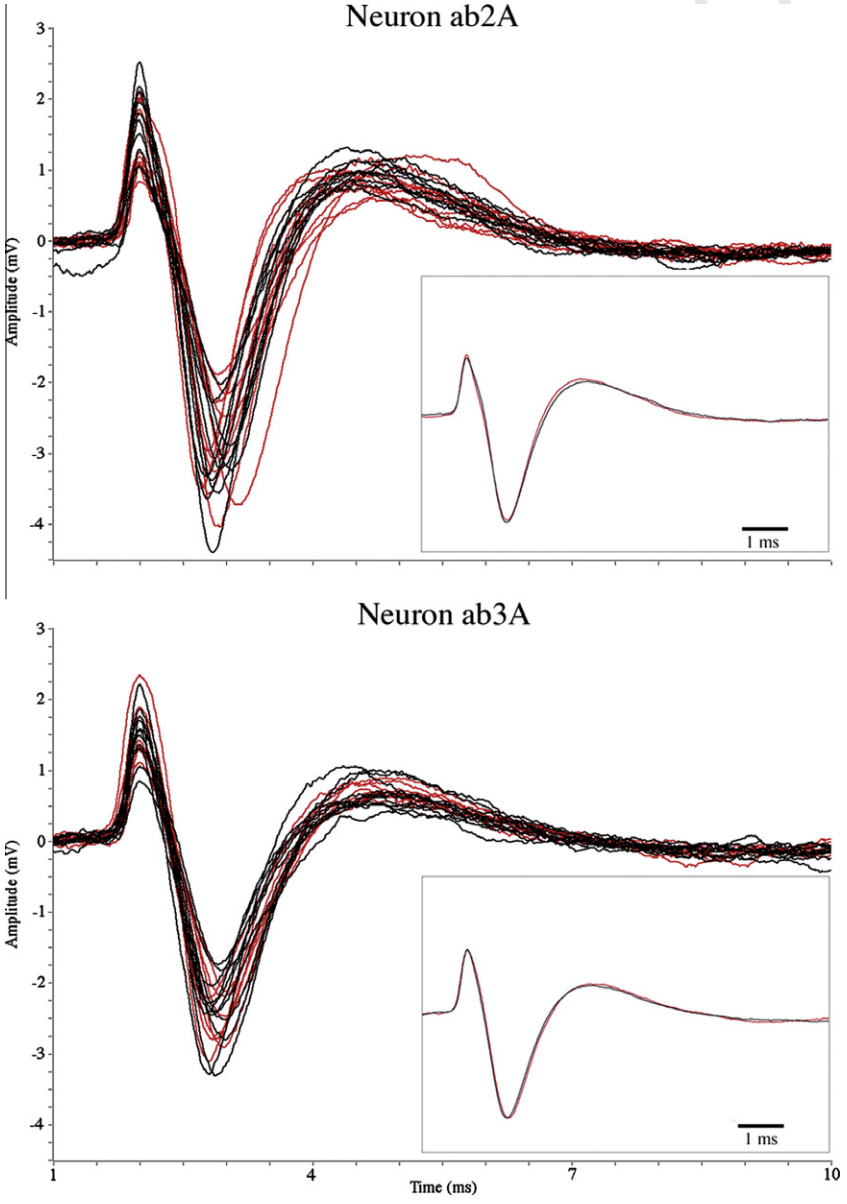

Fig. 7. Spontaneous spikes of heat-treated flies (EH) (red traces) and their controls (CH) (black traces) for neuron ab2A (top) and neuron ab3A (bottom). Insets: Mean traces of these spikes.

a pattern of activated glomeruli in the antennal lobe in the brain (Vosshall et al., 2000; Wang et al., 2003), and that information is used in the brain to drive behavioral responses after signal processing. But, because ORN activation mediates excitatory as well as

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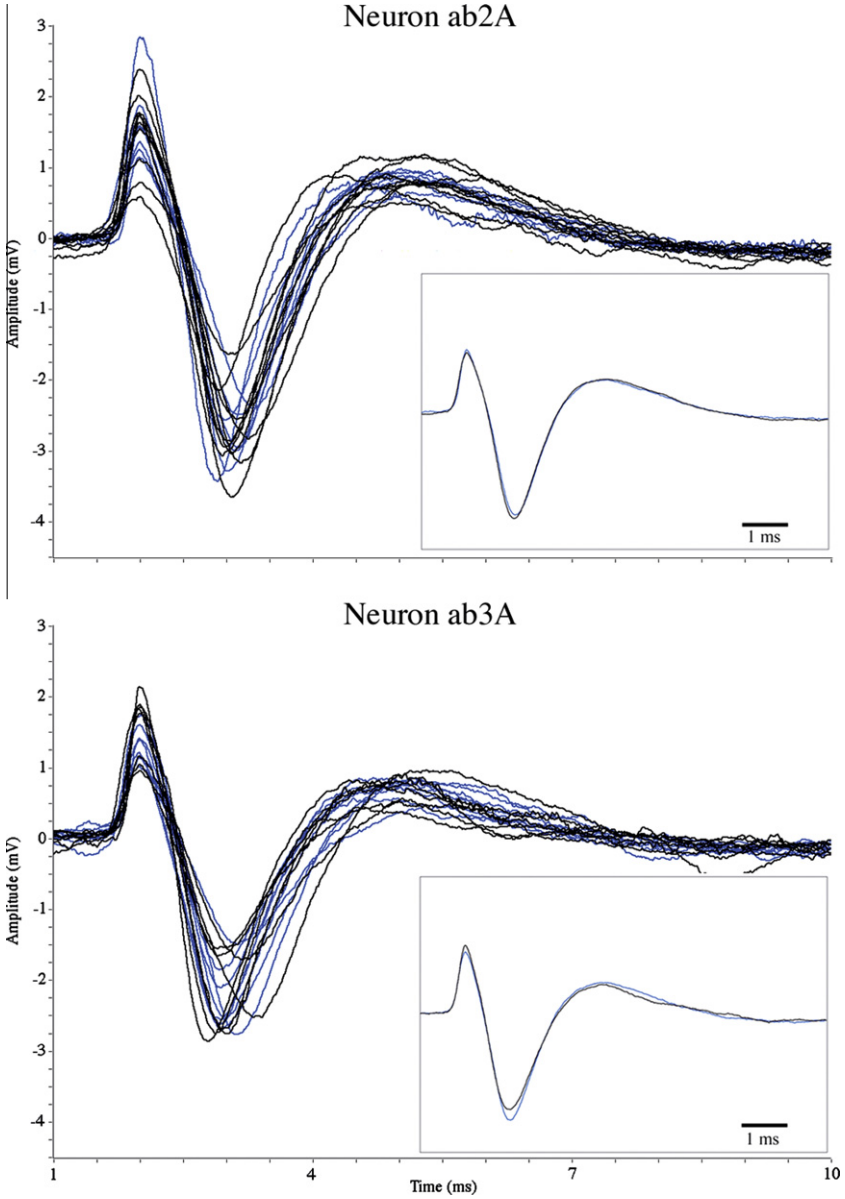

Fig. 8. Spontaneous spikes of cold-treated flies (EC) (blue traces) and their controls (CC) (black traces) for neuron ab2A (top) and neuron ab3A (bottom). Insets: Mean traces of these spikes.

inhibitory inputs in the antennal lobe the general level of olfactory activation at the antennae does not directly reflect odorant concentration.

At the antennal lobe of *D. melanogaster* selected glomeruli can mediate innate olfactory attraction or aversion (Semmelhack and Wang, 2009) and olfactory information integration may take into account the general level of activation at the antennal lobe (Borst, 1983). A recent study (Olsen et al., 2010) that correlates ORN signal input with PN (projection neuron) responses at the antennal lobe has shown in *Drosophila* that the activity of each neuron is normalized by activity in a larger pool of neurons by lateral inhibition, leading to some degree of concentration-invariant odor representations but preserving information about stimulus intensity while promoting a more efficient representation. This kind of normalization may provide a robust mechanism to overcome olfactory input variation linked to a continuously changing environment. Concentration invariant odor representations were also proposed previously for olfaction in *Drosophila* larvae (Asahina et al., 2009).

Future work on the molecular mechanisms responsible for the signal modulation described in this report may help to understand the actual processes activated in the antennae by temperature changes.

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