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The effect of environmental temperature on olfactory perception in *Drosophila melanogaster*

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ABSTRACT

Olfaction provides chemical information to an animal about its environment. When environmental conditions change, individuals should be able to adequately maintain function. Temperature may influence olfaction in a double manner, as it modifies the concentrations of gaseous compounds and affects biological processes. Here, we address acclimatization to environmental temperature in the olfactory system of *Drosophila melanogaster* using heat and cold treatments. Because the consequences of temperature shifts persist for some time after the treatment's end, comparison of olfactory behaviors at the same temperature in treated and untreated flies allows us to infer the biological effects of temperature in olfaction.

At intermediate odorant concentrations heat always generates a reduction of olfactory sensitivity, as they would be expected to compensate for the increase of volatiles in the air. Cold produces the opposite effect. These changes are observed in both sexes and in natural populations as well as in standard laboratory stocks.

Short applications suffice to cause detectable olfactory perception changes, but even prolonged temperature treatments have only a transitory effect. Together, these results suggest that olfaction in *Drosophila* underlies acclimatization to environmental temperature. However, sensitivity changes are not immediate and may cause imperfect adjustment of olfactory function for short time periods.

1. Introduction

In many species, olfaction is the primary sense that provides environmental information. In insects, for example, it is used to find food, mates and oviposition sites. Given the importance of the functions it performs, the system should be able to retain its ability to provide adequate information in the face of environmental changes.

Two main mechanisms serve to overcome temperature variations in nature; one involves thermosensation and mobility, which allows an organism to choose a habitat with the appropriate temperature range (McKemy, 2007), and the second mechanism is related to the ability of the animal to adapt to new conditions.

Numerous biological phenomena have been associated with fluctuations in temperature. In poikilothermic animals, body temperature shifts with changes in environmental temperature. In insects, body size and the length of the developmental period are shaped by temperature (Ashburner, 1989). It has also been shown that *Drosophila* populations undergo thermal adaptation in the laboratory (Laayouni et al., 2007) and in nature (Balanyá et al., 2006; Overgaard and Sørensen, 2008; Kristensen et al., 2008).

Various effects of temperature shifts have been reported. At the behavioral level, it has been shown for poikilothermic vertebrates that nervous system activity can partially compensate for temperature effects to preserve adequate function within a certain range of temperatures. At the cellular level, however, temperature fluctuations have a major impact on the functions of the nervous system and its components, inducing changes in conduction delay and synaptic gain (Montgomery and Macdonald, 1990). At the molecular level, several genes have been described that directly respond to heat and cold stresses, including the heat-shock-protein (*Hsp*) gene (Feder and Hofmann, 1999) and the cold-shock-protein (*Csp*) gene (Al-Fageeh and Smales, 2006).

Temperature may strongly influence olfaction, both by affecting biological processes (especially in small poikilothermic insects, in which the large surface-to-volume ratio results in a rapid thermal equilibrium with the ambient temperature (Zeiner and Tichy, 2000) and by modifying the gaseous odorant concentrations surrounding the animal.

Several reports have addressed the ability of the olfactory system to adapt to high odorant concentrations in the environment (Dalton, 2000). In *Drosophila*, adaptation to high concentrations of odorant has been described in larvae (Wuttke and Tompkins, 2000)

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and adults. In adults, prolonged exposure may induce structural changes in the central nervous system (Devaud et al., 2001, 2003). Short exposures to undiluted odorants diminish subsequent behavioral responses to the odorant. Electrophysiological signals from the antenna in response to short pulses of odorant also diminished; this is related to the function of TRP Ca^{2+} channels (Störtkuhl et al., 1999; Deshpande et al., 2000).

The global effects of environmental temperature on olfactory perception in the short term are not yet well understood. Temperature variations in nature constantly occur at different time scales, at different times of the day, and also when moving from one place to another (from sun to shade, for example). If environmental temperature variation is followed by olfactory system adjustment, do these changes compensate for the altered concentrations of odorants in the air? To answer this question, we investigated the effects of small temperature shifts at intermediate time scales (3–48 h). We either increased or decreased temperature (21 + 9 °C or 24 − 9 °C) in the range between 30 °C and 15 °C and measured the olfactory sensitivity of *Drosophila melanogaster*. These conditions are quite common in temperate climates. Since the consequences of heat or cold treatments persist for some time after the treatment's end, comparison of the olfactory behaviors of treated and untreated flies at the same temperature allowed us to infer the biological effects of temperature on olfactory sensitivity.

2. Materials and methods

2.1. Fly stocks

The wild-type Canton-S stock from the Bloomington Stock Center (Bloomington, Indiana, USA) was the only material used, except in one experiment. In the experiment performed to establish the general characteristics of the effect, we utilized the wild-type stock Lausanne-S (Bloomington, ID, USA) and two other natural populations captured in different locations in Asturias, Spain. P-1 and P-2 were each founded from more than 30 isofemale lines in 2004 and 2006, respectively.

2.2. Temperature treatments

In order to perform enough behavioral tests to completely characterize the possible effects of temperature, a maximum of 60 behavioral tests (Y-mazes) with 40 flies in each were carried out simultaneously. This set-up allowed us to apply temperature treatments during very precise time periods, but also prevented us from using behavioral paradigms like the T-maze, which requires continuous manipulation. T-mazes were therefore used only occasionally. Since the Y-maze measures olfactory preference over the course of 30 min, large temperature treatments (usually of 48 h) were initially chosen in order to maintain the effect throughout the test duration. The presence of the same temperature effect for shorter treatments was directly addressed in a subsequent experiment.

Fig. 1 depicts the different temperature treatments applied to the flies before the behavioral tests. The basic protocol for heat or cold treatments used in most experiments is shown in Fig. 1A. For the heat treatment experiments, the control (CH) and experimental (EH) groups differed only in the heat period (H), where flies cultured at 21 °C were subjected to 30 °C conditions for a certain time period. Afterward, flies were maintained at the test temperature (24 °C) for 90 min prior to the behavioral tests (for acclimation purposes). The duration of the acclimation period was determined by the results of the preliminary experiments described in supplemental material (S1 and S2). The duration was dependent upon the time necessary for our experimental conditions stabilize the flies' biology and to reach the environmental temperature inside the tubes containing the flies. After this time, we could assume that body temperature would remain constant during the 30-min Y-maze test. Thus, flies completing the maze in the first few minutes could be considered similar to those responding more slowly. The same principles were applied to the cold treatment protocol, in which the experimental group (EC) cultured at 24 °C was subjected to a 15 °C cold period (C).

In order to evaluate the durability of the effect produced by the temperature treatment, a second protocol was used (as depicted in Fig. 1B). The new IH and IC groups (for heat or cold treatments, respectively) incorporated an intermediate extinction period after temperature treatment.

Fig. 1C depicts the heat treatment applied in the T-maze experiments. In this case, no acclimation time was incorporated due to the short duration of the behavioral tests (1 min) and to the fact that flies were moved to a space other than the tube where they received temperature treatments prior the test. Thus, this protocol was developed to show the short-term effects of the temperature treatment.

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 protocols. The medium is nearly odorless, and therefore did not differentially affect the two groups.

2.3. Behavioral tests

Two different behavioral paradigms have been used to test olfactory preference: the Y-maze and the T-maze. For most experiments, a double choice, horizontally placed Y-maze was selected (Alcorta and Rubio, 1989; Martin et al., 2002). During the 30-min experiment, 40 females (except where otherwise indicated) that had been starved for 24 h chose between two tubes: a stimulus tube containing filter paper soaked with 0.5 ml of a certain concentration of odorant, and a control tube with 0.5 ml of solvent.

The second assay, the T-maze (Helfand and Carlson, 1989), was also a double-choice olfactory preference test. In this case, 30 flies were introduced into a central chamber in the sliding vertical plate from the start compartment. Once the plate was slid into the bottom position, flies could choose between the left and right sides during the 1-min experiment. One side was connected to a tube containing a filter paper soaked with the odorant at a certain concentration, and the other side was connected to a tube containing the solvent. This assay was performed in complete darkness.

An olfactory index (OI) was calculated by the number of flies in the stimulus tube divided by the total number of flies reaching the end of the maze at either end. This index measures olfactory preference from the total number of flies that chose one of the arms in the Y-maze, and disregards those flies that do not move from the initial tube. The choice of such an index, which is dependent only on the number of moving flies, is especially important in this report since temperature could directly affect the mobility of the flies.

OI values ranged from 0 (maximum repulsion) to 1 (maximum attraction), with the threshold of indifference at 0.5. Twenty replicate tests were performed for each line and stimuli. The number of replicate tests was increased in cases where differences were at the limit of statistical significance.

2.4. Odorants

Responses to different concentrations of ethanol and acetone in water and ethyl acetate and benzaldehyde in paraffin oil were examined. All chemicals were obtained from Merck (Darmstadt, Germany). All of these compounds are known to be odorous stimuli for *D. melanogaster*.