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RESEARCH ARTICLE

Food-derived volatiles enhance consumption in *Drosophila melanogaster*

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ABSTRACT

Insects use multiple sensory modalities when searching for and accepting a food source, in particular odor and taste cues. Food-derived odorants are generally involved in mediating long- and short-range attraction. Taste cues, in contrast, act directly by contact with the food source, promoting the ingestion of nutritious food and the avoidance of toxic substances. It is possible, however, that insects integrate information from these sensory modalities during the process of feeding itself. Here, using a simple feeding assay, we investigated whether odors modulate food consumption in the fruit fly *Drosophila melanogaster*. We found that the presence of both single food-derived odorants and complex odor mixtures enhanced consumption of an appetitive food. Feeding enhancement depended on the concentration and the chemical identity of the odorant. Volatile cues alone were sufficient to mediate this effect, as feeding was also increased when animals were prevented from contacting the odor source. Both males and females, including virgin females, increased ingestion in the presence of food-derived volatiles. Moreover, the presence of food-derived odorants significantly increased the consumption of food mixtures containing aversive bitter compounds, suggesting that flies integrate diverse olfactory and gustatory cues to guide feeding decisions, including situations in which animals are confronted with stimuli of opposite valence. Overall, these results show that food-derived olfactory cues directly modulate feeding in *D. melanogaster*, enhancing ingestion.

KEY WORDS: Olfaction, Feeding, Fruit fly, Sensory integration, Taste, Behavior

INTRODUCTION

Insects, like all animals, use sensory cues of different modalities to find food, mates, conspecifics and shelters, and to avoid predators. In particular, the chemosensory cues olfaction and taste play critical roles during seeking and identifying food, ultimately allowing insects to distinguish nutritious from potentially toxic food sources.

Although there is great variation in feeding habits and lifestyles, all insects have highly developed sensory structures that detect volatile (i.e. odors) or non-volatile (i.e. tastants) chemical cues. Odors are detected via specialized olfactory receptor neurons (ORNs) located on the antenna and in some insects also in the maxillary (in Diptera) or labial (in Lepidoptera) palps (Chapman, 1998; Hansson, 1995; Stocker, 1994). Many ORNs respond to only one or a few chemically related odorants, particularly when tested at behaviorally relevant and naturally occurring concentrations (Hallem and Carlson, 2006; Hansson et al., 1999; Hillier and Vickers, 2007; Røstelien et al., 2000; Stranden et al., 2003), while others are more broadly tuned (de Bruyne et al., 1999; Hallem and Carlson, 2006; MacKay et al., 2015; Yao et al., 2005).

Food tastants, in contrast, are detected by contact with gustatory receptor neurons (GRNs) found on several appendages and body parts including the proboscis labella, leg tarsi, pharynx, ovipositor and margin of the wings (Chapman, 1998; Falk et al., 1976; Montell, 2009; Stocker, 1994). GRNs respond to different taste qualities, named by analogy with human perception, including sweet, bitter, salt and water (Cameron et al., 2010; Hallem et al., 2006; Liman et al., 2014; Scott, 2018; Weiss et al., 2011).

Hungry insects, like all animals, are highly motivated to eat and actively search for food sources. Food-derived olfactory signals initiate the process of food searching and mediate long-distance attraction and orientation. For instance, pollinators follow floral odor plumes to locate distant flowers (Cardé and Willis, 2008; Riffell et al., 2014); mosquitoes and other blood-sucking insects come into the vicinity of a host solely through attraction to skin-derived odors and CO_2 (Guerenstein and Lazzari, 2010; Lehane, 2005; Ray, 2015; Takken and Knols, 1999). Once insects locate a potential food source, however, taste cues act by contact mediating the recognition, and ultimately promoting and sustaining the ingestion, of appropriate substances. In general, activation of sweet GRNs signals the presence of nutritious, caloric food, and starts a feeding program which involves proboscis extension and ingestion (Dethier, 1976; Gordon and Scott, 2009). Bitter substances are in general toxic and/or noxious (Chapman, 2003; Glendinning, 2002), and activation of bitter GRNs produces proboscis retraction, preventing the consumption of harmful substances (Dethier, 1976; French et al., 2015; Wang et al., 2004).

Olfactory and taste cues are not solely used sequentially during food searching and finding; insects can also integrate information from these two sensory modalities at or near the food source for learned associations. For instance, insects such as bees, moths and flies can readily learn to associate a neutral odor with a food reward (Bitterman et al., 1983; Chabaud et al., 2006; Daly and Smith, 2000; Giurfa and Sandoz, 2012; Kim et al., 2007). Thus, when the association is formed, the sole presentation of the odor stimulus elicits proboscis extension (Bitterman et al., 1983), a behavioral readout of feeding initiation. In addition to behaviors mediated by experience, proboscis extension can be innately enhanced by the presence of both odors and taste cues. For example, in the blowfly *Phormia regina*, the probability of proboscis extension upon contact with an appetitive food source increases in the presence of appetitive odors (Maeda et al., 2014) and can be affected by prior olfactory experience (Maeda et al., 2015). Similarly, in the fruit fly *Drosophila melanogaster*, odorants detected through

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the maxillary palps can enhance proboscis extension (Shiraiwa, 2008). Although these studies show that taste and olfactory information are integrated when animals attempt to eat, it is not known whether odors directly influence food consumption. Thus, in this work, using a simple behavioral assay, we tested whether the presence of food-derived odors modulates food consumption in *D. melanogaster*. Moreover, by pairing aversive taste cues with attractive olfactory cues, we examined how animals process such conflicting information to guide feeding decisions.

MATERIALS AND METHODS

Animals

Drosophila melanogaster, strain Canton-S, was used throughout; *D. melanogaster* strain 14021-0231.199, obtained from the University of California San Diego stock center, was also used in one experiment. Flies were grown on standard fly food at room temperature, in the absence of any of the odors used in experiments described below. Flies were 2–3 days old at the time of the experiments.

Proboscis extension response (PER) assay

The PER of starved mated female flies was tested in the presence or absence of banana volatiles. After 22 h of wet starvation, flies were gently anesthetized under CO_2 and singly glued by their dorsal thorax onto a wooden toothpick using clear nail polish, and then transferred to a humid chamber for 2 h at room temperature. Individual toothpicks with glued flies were mounted on a piece of Plasticine under the dissecting microscope; each fly was water satiated prior to testing.

A 1 ml plastic syringe coupled to Tygon tubing (3.2 mm internal diameter), with its outlet approximately 2 cm from the fly's head, delivered a constant flow of air. A piece of banana (1.5 g, or 1.5 g of wet cotton as control) was placed in a 5 ml syringe with a needle, and its tip was inserted inside the constant air stream tubing. Fifteen seconds after the constant airstream was turned on, the odor or control stimulus was injected into the airstream using manifold valves. After 30 s, the fly's tarsi were stimulated 3 times with increasing concentrations of sucrose solution (10, 50, 100 and 250 mmol l^{-1}; 20 s interval between concentrations). Each fly was stimulated with either odorous or clean air, and we recorded whether the fly extended its proboscis in response to each sucrose concentration (only full extensions were recorded). An inverted computer fan was placed near and behind the preparation to generate a steady air current over the fly, and for removing odors at the end of each trial.

Food consumption assay

Groups of 2 day old mated female flies (n=11–15 per vial) were wet starved for 24 h by placing each group in a fly vial containing two pieces of water-saturated Kim wipes. Flies were then transferred to a vial containing a piece of filter paper (2.7 cm diameter, Whatman, cat. no. 1001 125) impregnated with food solution (180 µl of 50 mmol l^{-1} D-glucose) dyed blue with Erioglaucine (0.25 mg ml^{-1}, Sigma-Aldrich) (Fig. 1A). To facilitate feeding, vials were flipped upside down so that the filter paper impregnated with food solution was at the top (Fig. 1A) (Jourjine et al., 2016). Flies were allowed to feed for 10 min and then vials were frozen for

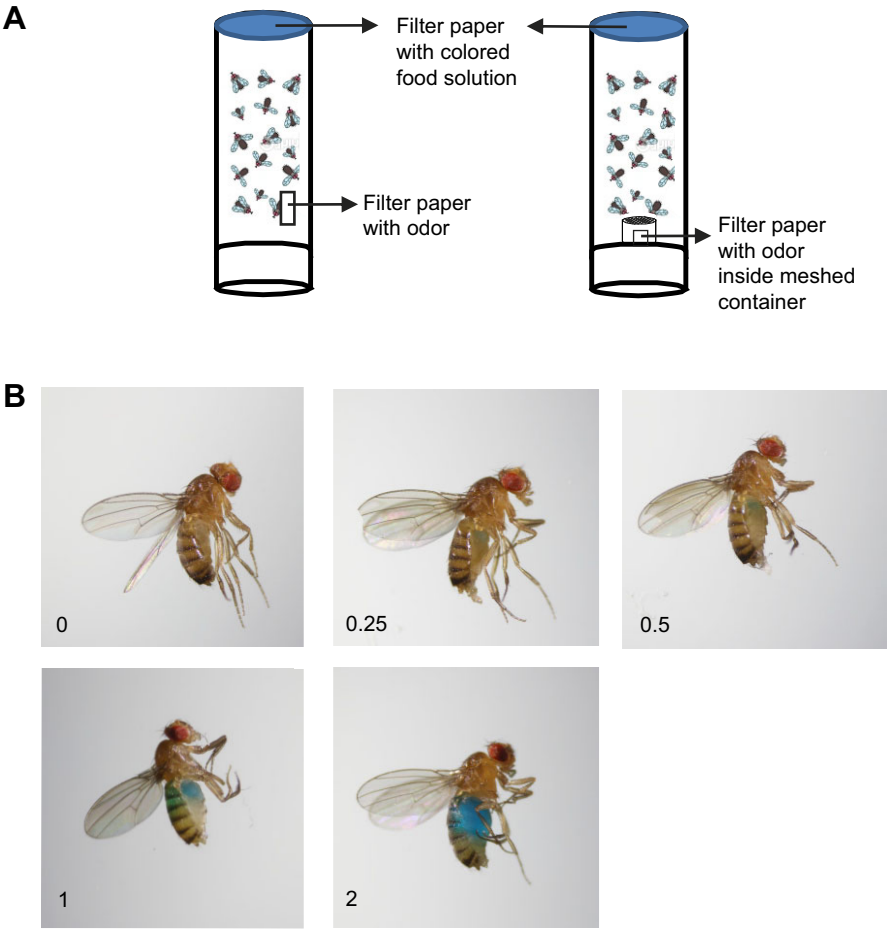

Fig. 1. Behavioral assay used to quantify feeding in the presence or absence of odors. (A) Groups of 10–15 wet-starved flies were transferred to a vial containing a disk of filter paper impregnated with food solution dyed blue and a strip of filter paper impregnated with an odorant or the solvent control; vials were immediately flipped upside down to facilitate feeding. Flies could contact the odor source (left) or not (right). (B) Representative examples of flies that consumed different amounts of food, visualized as blue dye. Consumption was quantified using a five-point scale ranging from 0 to 2 (shown in the individual images), as explained in the main text. Flies in each vial were scored blind to treatment, and a single score per vial was calculated and used for analysis.

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at least 60 min. After freezing, food consumption was measured by individually examining flies under a dissecting microscope and scoring them according to the relative amount of blue dye (i.e. food) in their abdomen (see 'Data analysis and statistics', below; Fig. 1B). All experiments and scoring were conducted blind to treatment.

In the first set of experiments, mated female flies were transferred to food vials containing an odor source. The odor source consisted of a small strip of filter paper (0.25 cm wide×1.5 cm long) impregnated with either 10 µl of an odor solution or 10 µl of the solvent as a control (mineral oil, Sigma-Aldrich, CAS no. 8042-47-5), placed 7 cm from the food source (Fig. 1A). The odor solution was loaded onto the filter paper 5–10 min before flies were transferred to the vial to allow volatile diffusion and stabilization. The odors used were ethyl acetate (Fisher Scientific, CAS no. 141-78-6), isoamyl acetate (MP Biomedicals, CAS no. 123-92-2) and methyl hexanoate (Sigma-Aldrich, CAS no. 106-7-7). All odors were diluted 1:10 and 1:20 v/v in mineral oil. Apple cider volatiles were also used in one experiment (20 µl of vinegar loaded onto filter paper, or 20 µl of distilled water as a control).

To prevent animals from contacting the odor source, a piece of filter paper impregnated with either 10 µl of mineral oil or 10 µl of odor solution, as above, was placed inside a 1.5 ml Eppendorf tube with its bottom cut off and replaced with a piece of plastic mesh affixed to the vial flug (Fig. 1A).

The consumption of food containing the aversive bitter compounds berberine (Sigma, CAS no. 633-65-8) or L-canavanine (Sigma, CAS no. 543-39-4) was tested in the presence or the absence of odorant volatiles. Flies were offered the following food mixtures (180 µl of food dyed blue loaded onto filter paper): (1) 50 mmol l^{-1} glucose only (control); (2) 50 mmol l^{-1} glucose+1 mmol l^{-1} berberine; and (3) 50 mmol l^{-1} glucose+10 mmol l^{-1} L-canavanine. All three mixtures were offered in the presence of isoamyl acetate 1:20 v/v or the solvent (mineral oil) control. As before, flies were prevented from contacting the odor source. These bitter compounds were chosen because they cause feeding aversion and/or strongly activate bitter receptors in *D. melanogaster* (French et al., 2015; Mitri et al., 2009; Weiss et al., 2011). All six groups of flies were tested with overlapping cohorts to allow direct comparisons between treatments.

For all experiments, control tests (i.e. in the absence of odors) were always conducted in parallel with experimental tests to control for day-to-day variations in feeding; excess control data were pseudo-randomly eliminated.

Data analysis and statistics

PER experiments

The number of animals showing proboscis extension for each sugar concentration in the presence or absence of banana volatiles was compared using Fisher's exact tests (Zar, 1999).

Feeding assay

After feeding and freezing, flies in each vial were individually scored using the following five-point scale based on the relative amount of food consumed, visualized as the level of blue dye in the abdomen: 0 (no dye=no food consumed), 0.25 ('trace' of blue dye='taste' of food), 0.5 (up to ¼ of the abdomen dyed blue=some feeding), 1 (more than ¼ but less than ½ of the abdomen dyed blue=moderate feeding) and 2 (more than ½ of the abdomen dyed blue=extensive feeding) (Fig. 1B). For each vial, a single feeding score was calculated as: $(0\times n_0+0.25\times n_{0.25}+0.5\times n_{0.5}+1\times n_1+2\times n_2)/N$, where $n_{(0–2)}$ denotes the number of flies in each score category, and N is the total number of flies per vial. This feeding score was modified from a previously published one (Jourjine et al., 2016) and provided greater sensitivity and resolution.

Data from each day were normalized (feeding in the presence of odor/feeding in the absence of odor) for comparisons involving flies from different cohorts and sexes. Control data for each day and condition were averaged and this average value was used for normalization.

Data from the first experiment were initially analyzed using two-way ANOVA, but the interaction between factors (odorant and concentration) was significant, thus precluding interpretation of the main factor effects (Zar, 1999). Therefore, data from this experiment were analyzed using Kruskal–Wallis tests for each of the two concentrations used. Significant results were followed by Dunnett's test (for comparisons involving equal sample sizes) or Dunn's test (for comparisons involving unequal sample sizes) to compare control versus experimental groups. The effect of concentration for each odor was analyzed using two-tailed Mann–Whitney tests. In most cases, sample sizes were increased in tests with non-significant results to achieve enough statistical power (Zar, 1999). In other experiments, Mann–Whitney tests (two-tailed) or Kruskal–Wallis tests were used for respectively comparing two or more than two means (Zar, 1999). Statistical analyses were conducted using SigmaPlot v.13 (Systat).

RESULTS

Effects of odors on PER

A previous study reported that odors increase PER in *D. melanogaster*, but only animals with their antennae or maxillary palps severed were tested (Shiraiwa, 2008). Therefore, we first confirmed that under our experimental conditions the presence of food-derived odors increases the probability of PER in intact animals. We observed a significant enhancement of PER in the presence of banana volatiles (Fig. 2) only at the 50 mmol l^{-1} concentration, consistent with previous reports (Shiraiwa, 2008). We therefore chose this sugar concentration for all feeding assays.

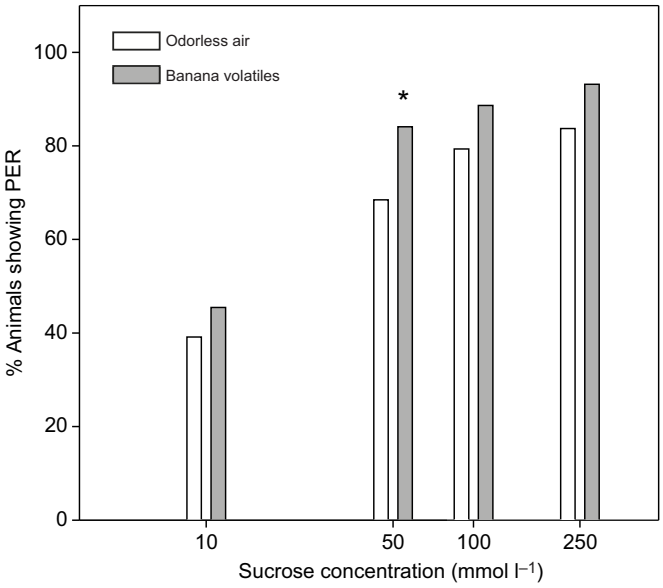

Fig. 2. Food-derived odors increase the probability of proboscis extension. Percentage of animals that extend their proboscis for each sucrose concentration (log scale) in the absence (white bars, n=104) or presence (gray bars, n=100) of banana volatiles. Each animal was tested with all sugar concentrations but with either the control or odor stimuli. The asterisk indicates significant differences (Fisher's exact test, P<0.05).

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Effects of odors and odor-derived volatiles on glucose consumption

In order to test whether odors increase food consumption, we selected compounds which are known attractants of *D. melanogaster*, are found within food sources and elicit strong responses from ORNs (Christiaens et al., 2014; de Bruyne et al., 2001; Faucher et al., 2013; Hallem and Carlson, 2006; Pelz et al., 2006; Schubert et al., 2014; Stensmyr et al., 2003). Overall, we found that flies ate more in the presence than in the absence of food-related odors, and that the enhancing effect of odors depended on both the identity of the odorant and its concentration (Fig. 3). Flies ate significantly more in the presence of ethyl acetate at the 1:10 v/v concentration, and in the presence of either isoamyl acetate or methyl hexanoate at the 1:20 v/v concentration (Fig. 3; Kruskal–Wallis tests followed by *post hoc* tests). Isoamyl acetate at 1:20 v/v had the largest effect on food consumption, with a median feeding score 2.4 times higher than that of control flies. Feeding scores were significantly affected by the concentration of both ethyl acetate and isoamyl acetate (Fig. 3; $P<0.05$, Mann–Whitney tests) but not by the concentration of methyl hexanoate ($P>0.05$).

To test whether contact with the odor is required for increased consumption, we exposed flies to odor volatiles but prevented them from contacting the odor source. We used isoamyl acetate 1:20 v/v as this odorant/concentration produced the strongest effect (Fig. 3). Female flies ate significantly more in the presence than in the absence of isoamyl acetate (Fig. 4A; Mann–Whitney tests, $P<0.05$). In addition, the normalized feeding scores of flies that could contact the odor source (data from the previous experiment) were not different from those of flies that were not able to do so (Fig. 4B; Mann–Whitney test, $P>0.05$). Thus, these results demonstrate that volatile (i.e. olfactory) cues are necessary and sufficient to enhance feeding, and that contact with the odor does not significantly contribute to this effect. Moreover, we found that volatile cues increased food consumption in a different strain of *D. melanogaster* (Fig. 4C).

We also studied whether enhancement of feeding by volatiles is affected by other important variables such as sex, female mating status and odor complexity. As observed in mated females, mated males ate significantly more in the presence than in the absence of volatile cues (Fig. 5A; Mann–Whitney tests, $P<0.05$). Although males ate significantly more than females whether in the presence or absence of volatiles (Mann–Whitney tests, $P<0.05$), their normalized feeding scores were not statistically different from each other (Fig. 5B; Mann–Whitney test, $P>0.05$), indicating that food-derived olfactory cues similarly enhance feeding in both sexes. Odor-derived volatiles also increased food consumption in virgin females. The median feeding scores of virgin females fed in the presence of isoamyl acetate volatiles were 1.5 times (at 1:10 v/v) and 1.3 times (at 1:20 v/v) higher than those of control flies (Fig. 5C). This increase was statistically significant only at the 1:10 v/v concentration (Kruskal–Wallis test followed by Dunnett's test, $P<0.05$). Control (i.e. tested in the presence of mineral oil) mated and virgin females ate similar amounts of food (Mann–Whitney test, $P>0.05$). In the presence of isoamyl acetate 1:20 v/v, their normalized feeding scores were not statistically different (Mann–Whitney test, $P>0.05$; Fig. 5D). Mated females offered glucose in the presence of apple cider volatiles ate significantly more than control flies (Mann–Whitney test, $P<0.05$; Fig. 4D), indicating that odorant mixtures enhanced feeding as well.

Effect of volatiles on the consumption of aversive food mixtures

Under natural situations, animals are often confronted with stimuli of conflicting valence that predict opposite behavioral outcomes

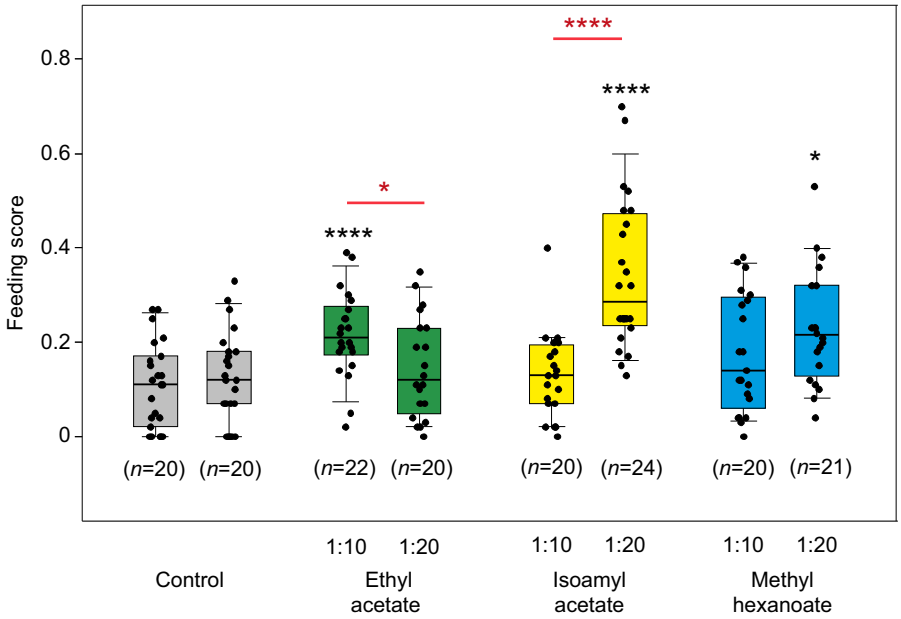

Fig. 3. Food-derived volatiles enhance food consumption. Feeding scores of flies offered 50 mmol l^{-1} glucose in the presence or absence of ethyl acetate, isoamyl acetate and methyl hexanoate at 1:10 v/v and 1:20 v/v concentration. Boxplots indicate the median feeding score (horizontal line within the box), the 25th and 75th percentiles (lower and upper margins of the box), and the 10th and 90th percentiles (whiskers); circles show individual data points. Control flies (n=40 vials randomly divided into two groups) were offered food in the presence of the mineral oil solvent. Flies could contact the odor source (as shown in Fig. 1A, left); all groups were tested with overlapping cohorts. Numbers in parentheses indicate the number of vials tested for each odor and concentration. Data for each odor concentration were analyzed using Kruskal–Wallis tests (d.f.=3); significant results were followed by Dunn's test, comparing each odor with the mineral oil control group (black asterisks; *$P<0.05$, ****$P<0.001$). For each odor, the effect of concentration was analyzed using two-tailed Mann–Whitney tests (red horizontal lines and asterisks; *$P<0.05$, ****$P<0.001$). All odors enhanced feeding above the control level at one or the other concentration used. Isoamyl acetate at 1:20 v/v had the strongest effect on feeding enhancement.

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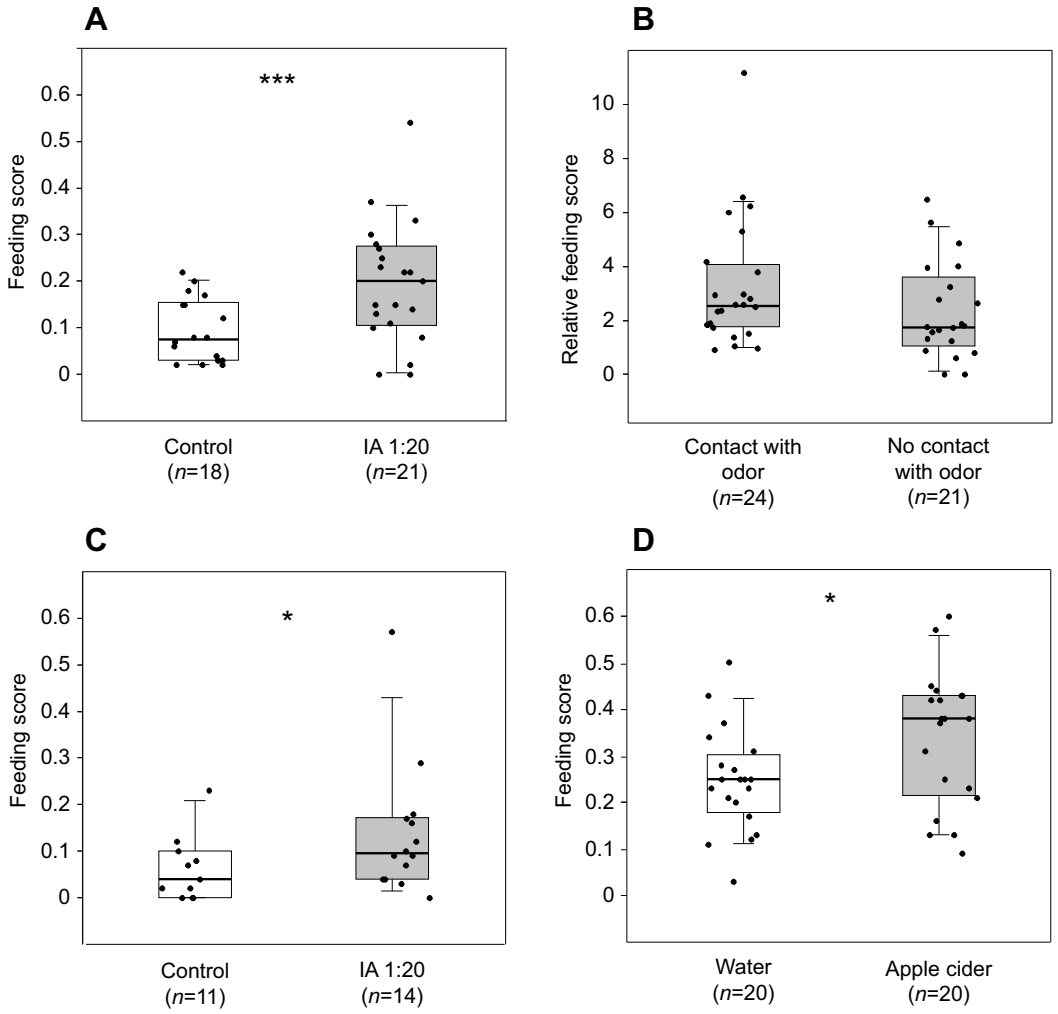

Fig. 4. Contact with the odor source is not required to enhance food consumption. (A) Feeding score of mated Canton-S female flies offered 50 mmol l^{-1} glucose in the presence or absence (control, mineral oil) of isoamyl acetate (IA) 1:20 v/v (loaded on filter paper); flies could smell but could not contact the odor source (as shown in Fig. 1A, right). Differences were statistically significant (P<0.005, two-tailed Mann–Whitney test). (B) Relative feeding scores of Canton-S flies offered food in the presence of isoamyl acetate volatiles with (left) without (right) the ability to contact the odor cue. For each vial, the relative feeding score was calculated by dividing the feeding score in the presence of the odor by the average feeding score of control flies (two-tailed Mann–Whitney test, P>0.05). (C) Feeding scores of mated females from a different strain of *D. melanogaster* (14021-0231.199, abbreviated as 199) offered 50 mmol l^{-1} glucose in the presence or absence of isoamyl acetate volatiles (*P<0.05, two-tailed Mann–Whitney test). As with Canton-S flies, volatile cues were sufficient to enhance feeding. (D) Feeding scores of mated female Canton-S flies offered 50 mmol l^{-1} glucose in the presence or absence of apple cider volatiles (40 µl loaded on filter paper or 40 µl of water). As with single odorants, complex odor mixtures can also enhance feeding. In all cases, boxplots indicate the median feeding score (horizontal line within the box), the 25th and 75th percentiles (lower and upper margins of the box), and the 10th and 90th percentiles (whiskers); circles show individual data points.

(e.g. Klappenbach et al., 2017; Lewis et al., 2015). In particular, natural food sources are mixtures that might contain substances which are not appetitive by themselves, including bitter compounds, such as alkaloids in nectar from some flowers (Adler, 2000) and glucosinolates in *Brassica* (Cartea and Velasco, 2008). Thus, a plausible hypothesis is that odorants promote, to some extent, ingestion of such food mixtures. We tested this idea by investigating whether the presence of food-derived volatiles enhances feeding on food sources which are normally rejected or less accepted, such as sweet–bitter mixtures (Chapman et al., 1991; French et al., 2015; Meunier et al., 2003). Mated females were offered 50 mmol l^{-1} glucose alone, 50 mmol l^{-1} glucose+1 mmol l^{-1} berberine, or 50 mmol l^{-1} glucose+10 mmol l^{-1} L-canavanine, in the presence or absence of isoamyl acetate volatiles. These two bitter compounds were selected because they have different influences on GRN activity: berberine activates bitter GRNs and inhibits sugar GRNs, whereas L-canavanine activates bitter GRNs but does not inhibit sugar GRNs (French et al., 2015; Jeong et al., 2013). Food mixtures containing either bitter substance significantly reduced feeding (Fig. 6; P<0.05 in both cases, Kruskal–Wallis tests followed by Tukey tests).

In order to directly test the effect of volatiles on the consumption of food containing bitter compounds, we compared consumption of each food source in the presence or absence of the odor. As before, the presence of isoamyl acetate significantly increased consumption of glucose (Fig. 6; Mann–Whitney test; P<0.05): the median feeding score of flies in the presence of isoamyl acetate was 2.1 times higher than that of flies fed in the presence of the solvent control. The feeding enhancement effect of isoamyl acetate was even stronger when flies were offered food mixtures containing bitter substances. The presence of isoamyl acetate significantly increased the median consumption of food mixtures containing

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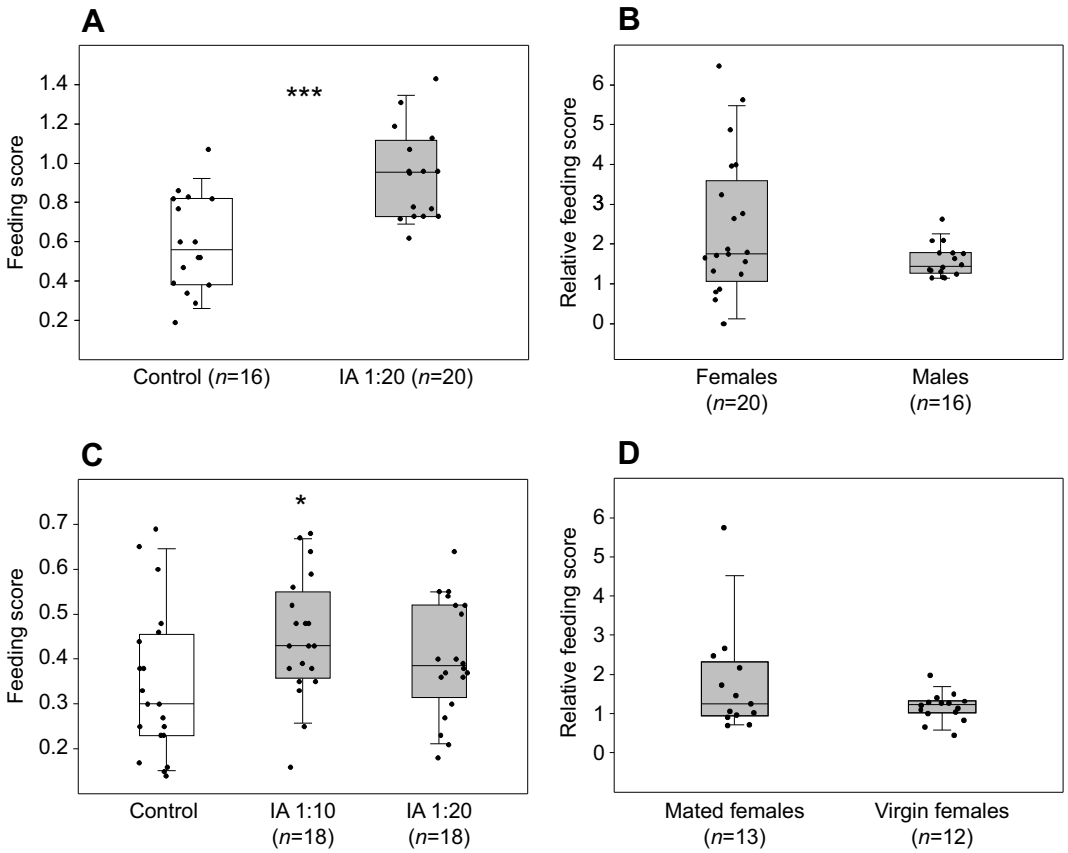

Fig. 5. Odorant volatiles enhance feeding in mated males and virgin females. (A) Feeding scores of mated male flies offered 50 mmol l^{-1} glucose in the presence or absence (control, mineral oil) of isoamyl acetate (IA) volatiles (1:20 v/v loaded on filter paper) (***P<0.005, two-tailed Mann–Whitney test). (B) Relative feeding scores of Canton-S female flies (from Fig. 4A) and mated males (from A). For each vial, the relative feeding score was calculated by dividing the feeding score in the presence of the odor by the average feeding score of control flies (no odor) from the same cohort. Differences were not statistically significant (two-tailed Mann–Whitney test, P=0.08). (C) Odor volatiles enhanced feeding in virgin female flies at 1:10 v/v concentration (Kruskal–Wallis test followed by Dunnett's test, *P<0.05). (D) Isoamyl acetate 1:20 v/v similarly enhance feeding in mated and virgin females (Mann–Whitney test, P>0.05). Only flies from the same cohort were included in this analysis. In all cases, boxplots indicate the median feeding score (A,C) or normalized feeding score (B,D) (horizontal line within the box), the 25th and 75th percentiles (lower and upper margins of the box), and the 10th and 90th percentiles (whiskers); circles show individual data points.

berberine or L-canavanine (3.1 and 5.5 times, respectively) (Fig. 6; Mann–Whitney tests, P<0.05 in both cases). Overall, these results show that odors increase food consumption, both for appetitive food and for food mixtures containing bitter compounds.

DISCUSSION

We investigated the effect of attractive food-derived volatiles on food consumption in *D. melanogaster*. We found that the presence of food-derived volatiles increased ingestion in a concentration- and odorant-dependent manner, and that contact with the odor source is not required to produce this enhancement. When flies were offered food mixtures containing bitter compounds, they consumed less, but such mixtures were more readily accepted in the presence of odorant volatiles. Overall, these results indicate that flies integrate diverse olfactory and gustatory cues to guide food consumption.

In flies, studies of taste and feeding have extensively used the PER as a measure of taste palatability and appetite. When contact chemosensilla of either the tarsi or the proboscis are stimulated with an appetitive stimulus such as a sugar solution, insects extend their proboscis in an attempt to eat (Dethier, 1976). Thus, PER assesses the behavioral response to gustatory stimuli in the absence of consumption. In blowflies, the PER threshold to sucrose decreased in the presence of an odor positively associated with a food reward (Nisimura et al., 2005). Also, odors detected through the maxillary palp increased the probability of PER in starved animals (Maeda et al., 2014; Shiraiwa, 2008).

First, we confirmed that complex food-derived volatiles increased the probability of PER in intact *D. melanogaster* (Fig. 2). We then directly tested whether food-derived odors increased food ingestion. All odorants tested enhanced feeding to different degrees, with isoamyl acetate having the strongest effect (Fig. 3). This odorant is prominent in the headspace of banana and elicits strong responses from antennal ORNs (Hallem and Carlson, 2006; Schubert et al., 2014). For a given odorant, the enhancing effect depended on the concentration, e.g. isoamyl acetate and methyl-hexanoate significantly increased feeding only at the lowest concentration tested, while ethyl acetate increased feeding only at the highest concentration (Fig. 3). This is in line with the fact that the same odorant or odor blend can trigger avoidance, indifference or attraction depending on the concentration (Semmelhack and Wang, 2009; Stensmyr et al., 2003). Collectively, these odorants strongly activate at least seven different olfactory receptors (ORs) expressed in the antenna (Or22a, Or85b, Or47a, Or42b, Or43b, Or10a and Or98a) and one OR (Or42a) expressed in the maxillary palps (Galizia et al., 2010; Hallem and Carlson, 2006; Münch and Galizia, 2016; Pelz et al., 2006). Our study does not allow us to infer which ORs mediate feeding enhancement, but two of the odorants tested (isoamyl acetate and methyl hexanoate) strongly activate both

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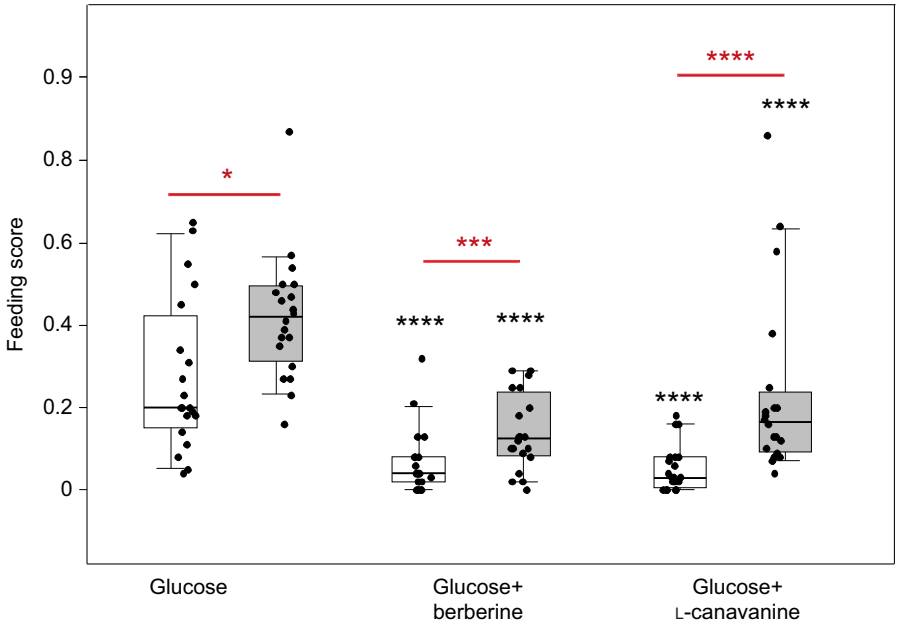

Fig. 6. Odorant volatiles enhance feeding on food sources containing bitter compounds. Feeding scores of flies offered (1) 50 mmol l^{-1} glucose, (2) 50 mmol l^{-1} glucose+1 mmol l^{-1} berberine, or (3) 50 mmol l^{-1} glucose+10 mmol l^{-1} L-canavanine in the absence (white boxes) or presence (gray boxes) of isoamyl acetate volatiles (1:20 v/v loaded on filter paper). All six groups of flies (n=20 vials in each group) were tested with overlapping cohorts. As shown before, volatiles enhanced feeding on appetitive (glucose only) food sources (P<0.05, two-tailed Mann–Whitney test). Whether in the presence or absence of volatiles, food mixtures containing either bitter compound significantly reduced feeding (Kruskal–Wallis tests followed by Dunnett's test, black asterisks; ****P<0.001). However, isoamyl acetate volatiles significantly increased the feeding scores of flies fed food mixtures containing the bitter compounds (two-tailed Mann–Whitney tests, red horizontal lines with asterisks; ***P<0.005, ****P<0.001). Boxplots indicate the median feeding score (horizontal line within the box), the 25th and 75th percentiles (lower and upper margins of the box), and the 10th and 90th percentiles (whiskers); circles show individual data points.

Or22a and Or85b, which are expressed in the antennae (Hallem and Carlson, 2006; Münch and Galizia, 2016; Pelz et al., 2006). Or22a may be of particular significance, as this OR is activated by odorants released by the host fruit of *D. melanogaster* (Mansourian et al., 2018) and of its close relatives *Drosophila sechellia* (Dekker et al., 2006) and *Drosophila erecta* (Linz et al., 2013). These findings thus suggest that feeding enhancement may proceed through the antenna but do not preclude the possibility that odors enhance ingestion through the maxillary palps as well. *Drosophila melanogaster* maxillary palps are highly sensitive to behaviorally relevant odorants and can mediate short- and long-range attraction (Dweck et al., 2016), and are involved in enhancing taste responses in this fly species (Shiraiwa, 2008).

We further confirmed that the enhancing effect of odors in food consumption is purely olfactory. Animals ingested significantly more in the presence than in the absence of isoamyl acetate volatiles (Fig. 4A) and consumed similar relative amounts of food with or without contact with the odor source (Fig. 4B). Thus, these results demonstrate that olfactory input is necessary and sufficient to increase ingestion. Odorant volatiles also enhanced feeding in a different strain of *D. melanogaster* (Fig. 4C), indicating that this effect is a generalized characteristic of this, and possibly other, fruit fly species.

We found that volatiles from apple cider vinegar, a food source that is highly attractive to flies (Becher et al., 2010; Semmelhack and Wang, 2009), also enhanced ingestion (Fig. 4D). The headspace of apple cider vinegar contains a variety of carbonyls, esters and alcohols, including isoamyl acetate and ethyl acetate (Aurand et al., 1966), both of which are sufficient in themselves to enhance food consumption (Figs 3 and 4). Thus, it is possible that components of an odor mixture are exploited differentially during the processes of food searching, finding and feeding. A blend of key odorants is usually required for long-distance orientation towards a food source (Becher et al., 2010; Riffell et al., 2009), although in a few cases single odorants maybe sufficient but much less effective (Becher et al., 2010; Dweck et al., 2016). Once insects are in the vicinity of the food source and contact an appetitive taste, single components within the odor mixture may be sufficient to enhance ingestion.

Certain odors and odorants, such as balsamic vinegar and ethyl acetate, induce stronger and longer olfactory responses in female than in male flies (Steck et al., 2012). We found, however, that isoamyl acetate similarly modulates feeding in the two sexes (Fig. 5A,B). This is not surprising, given that single odorants evoke activity in multiple ORs and antennal lobe glomeruli (de Bruyne et al., 2001; Hallem and Carlson, 2006; Hallem et al., 2004; Wang et al., 2003). Isoamyl acetate also enhanced feeding in virgin females but at a higher concentration than in mated females (Fig. 5C). A possible explanation for this difference is that virgin females may be less sensitive to isoamyl acetate than mated females, although considerable variation exists regarding the odor context and biological significance of the odorants (Gadenne et al., 2016). Wind tunnel experiments in *D. melanogaster* showed similar levels of attraction to vinegar volatiles in males and females (Becher et al., 2010), a complex mixture that includes isoamyl acetate.

Taste cues evoke stereotypical attractive or aversive behavioral responses, albeit these responses can be modified by the behavioral context and the insect's internal state. For instance, starved flies accept foods containing bitter compounds, a change that results from sensitization to sweet substances and desensitization to bitter compounds (Inagaki et al., 2014; LeDue et al., 2016; Meunier et al., 2003). Moreover, under natural situations, food sources may be composed of multimodal sensory stimuli of conflicting valence. We thus tested whether appetitive food-derived odorants enhance ingestion of food sources which are normally rejected or less accepted, such as sweet–bitter mixtures (Chapman et al., 1991; French et al., 2015; Meunier et al., 2003). As expected, addition of a bitter compound to an appetitive sweet stimulus strongly reduced feeding (Fig. 6, white bars). However, we found that the presence of attractive volatiles (isoamyl acetate) significantly increased consumption of these food mixtures (Fig. 6, gray bars). This enhancing effect was observed when mixtures included either of two different classes of bitter compounds, the alkaloid berberine or the legume amino acid L-canavanine. These two bitter compounds differ in their mechanism of feeding suppression, with L-canavanine activating bitter-sensitive cells and berberine shutting down taste responses in sugar-sensitive cells (French et al., 2015; Jeong et al., 2013). Although it remains to be investigated, these results, along with previous evidence (see discussion below), suggest that processing of such conflicting information, e.g. an aversive taste

cue and an appetitive odor stimulus, likely occurs in higher order brain centers.

Overall, our results show that food-derived odorants enhance food ingestion, although it remains to be investigated whether other meaningful odors increase consumption as well. What are the neural mechanisms underlying this effect? Importantly, the finding that odors increase the probability of proboscis extension in flies with intact olfactory organs indicates that the enhancement effect of odors on food consumption is independent of odor-evoked increases in locomotion activity (Jung et al., 2015). In blowflies, neuroanatomical evidence suggests that integration of taste and palp-mediated olfactory cues might take place in the subesophageal ganglion, as the terminals of some palp ORNs overlaps with those of GRNs in this region (Maeda et al., 2014). It is possible, however, that odors increase feeding through neural circuits in higher brain areas in *D. melanogaster*. The lateral horn of the protocerebrum and the mushroom bodies are two good candidates, as these regions receive input from antennal lobe olfactory projection neurons (Marin et al., 2002; Wong et al., 2002). Furthermore, two classes of second-order taste projection neurons project to the superior lateral protocerebrum (Kim et al., 2017), an area that provides inputs and outputs to the mushroom bodies (Ito et al., 1998), in close proximity to olfactory projection neurons in the lateral horn. The lateral horn of the protocerebrum is thought to mediate innate, stereotypical olfactory-driven behaviors, while the mushroom bodies are typically regarded as learning and memory centers (Heimbeck et al., 2001; Jefferis et al., 2007). The mushroom bodies have been extensively implicated in olfactory learning (Davis, 2005; de Belle and Heisenberg, 1994) but are also necessary for learned taste behaviors (Kirkhart and Scott, 2015; Masek and Keene, 2016); they are also involved in other complex functions including sensory integration (Balkenius and Balkenius, 2016; Farris, 2008; Yagi et al., 2016) and context recognition (e.g. Bräcker et al., 2013). Thus, a plausible hypothesis is that food odors enhance feeding through local circuits within these higher order brain regions. The mushroom bodies may also be involved in the simultaneous evaluation of aversive taste and attractive odor cues in the feeding context, as previous studies have shown that specific local circuits within these brain structures are required to integrate information about olfactory appetitive and aversive sources (Bräcker et al., 2013; Lewis et al., 2015). In sum, our results demonstrate that food-derived odorants can enhance consumption of appetitive substances in *D. melanogaster*, and this, along with the powerful genetic techniques available in this fly species, offers an excellent opportunity for studying the neural mechanism(s) underlying multisensory integration in the context of feeding.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
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