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RESEARCH ARTICLE

The sugar meal of the African malaria mosquito *Anopheles gambiae* and how deterrent compounds interfere with it: a behavioural and neurophysiological study

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SUMMARY

In this study, we show that female African malaria mosquitoes *Anopheles gambiae* starved for 3–5 h start to engorge on sucrose at concentrations between 50 and 75 mmol l^{-1}. Half of the feeding response (ED_{50}) is reached at 111 mmol l^{-1} and the maximum response (0.4 mg) occurs at 250 mmol l^{-1}. Two receptor cells in a trichoid sensillum of the labellum, called the 'sucrose' and 'water' neurones, are activated by sucrose and water, respectively. The electrophysiological response of the sucrose receptor cell starts well below the level of sugar necessary to induce engorgement. The sugar receptor cell is most sensitive to small increments in sucrose concentration up to 10 mmol l^{-1} with a response plateau from 25 mmol l^{-1}. Fructose has a mild phagostimulatory effect on *A. gambiae*, whereas no significant differences in meal sizes between water and glucose were found. However, when 146 mmol l^{-1} fructose plus glucose are mixed, the same engorgement as on 146 mmol l^{-1} sucrose is observed. Likewise, even though the sucrose receptor cell is not activated by either fructose or glucose alone, equimolar solutions of fructose plus glucose activate the neurone. We conclude that there is a behavioural and neurophysiological synergism between fructose and glucose, the two hexose sugars of sucrose. We show that some bitter-tasting products for humans have a deterrent effect on feeding in *A. gambiae*. When 1 mmol l^{-1} quinidine, quinine or denatonium benzoate is added to 146 mmol l^{-1} sucrose, feeding is almost totally inhibited. The effect of berberine is lower and no significant inhibition on engorgement occurs for caffeine. The deterrent effect depends on the concentration for both quinine and quinidine. Capillary feeding experiments show that contact chemosensilla on the mouthparts are sufficient for the detection of sucrose and bitter products. The feeding assay findings with deterrents correlate with the neurophysiological responses of the sucrose and water labellar neurones, which are both inhibited by the bitter compounds denatonium benzoate, quinine and berberine between 0.01 and 1 mmol l^{-1}, but not by the same concentrations of caffeine. In conclusion, sucrose stimulates feeding and activates the labellar sucrose neurone, whereas feeding deterrents inhibit both the sucrose and water neurones. This study provides an initial understanding of the physiological mechanisms involved in sugar feeding in *A. gambiae* and shows how some bitter products interfere with it.

Supplementary material available online at http://jeb.biologists.org/cgi/content/full/216/7/1292/DC1

Key words: *Anopheles gambiae*, malaria mosquito, insect gustation, taste neurone, nectar feeding, feeding deterrent.

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INTRODUCTION

Plant-derived sugar is the basic food of adult mosquitoes (Foster, 1995), usually including sucrose, which is also the most common sugar in nectar, along with fructose and glucose (Wykes, 1952; Baker and Baker, 1982; Van Handel, 1984; Petanidou and Lamborn, 2005; Manda et al., 2007b). Carbohydrates are consumed by males to supplement the energy required for their intense swarming (Yuval et al., 1994; Gary et al., 2009). Females of anautogenous mosquito spp. feed on sugar to provide energy for flight and increase longevity, but need blood to produce eggs (Gary and Foster, 2001). The major source of sugar is in floral nectaries, although mosquitoes also feed on extrafloral nectaries, honeydew, tree sap, damaged fruits and leaves or discarded plant material like sugar-cane waste (Foster, 1995). Glucose can also be found in vertebrate blood, although its calorific content is negligible compared with that of blood proteins (Foster, 1995). In the field, mosquitoes are generally observed to feed on flowers in full bloom that are abundant, demonstrating opportunism (Magnarelli, 1979). However, in the African malaria mosquito *Anopheles gambiae* (Giles), some clear preferences exist for particular plants available in the vicinity of human hosts or oviposition sites (Manda et al., 2007b). To date, the physiological basis in *A. gambiae* for discriminating between plant species is still unknown. At medium to long range, plant volatiles probably inform mosquitoes of the presence of a sugar source (Foster and Takken, 2004) as well as those of unsuitable plants, as shown by the strong repellent activity of some plant extracts against female *A. gambiae* searching for a host (Odalo et al., 2005). After landing on the plant, mosquitoes use terminal pore sensilla on their legs and mouthparts to find the nectar source (Pappas and Larsen, 1978) and probably to taste meal quality, such as the sugar concentration or the presence of toxic and unpalatable substances. Behavioural avoidance of noxious allelochemicals is a mechanism of defence developed by insects against plant toxins. Deterrence (feeding inhibition) by bitter-tasting plant secondary metabolites such as alkaloids is known in phytophagous insects such as the moth *Manduca sexta* (Glendinning, 2002), in the scavenger fly *Phormia regina* (Dethier and Bowdan, 1992) and in the fruitfly *Drosphila melanogaster* (Meunier et al., 2003). Recently, it has been shown that the alkaloids quinine and caffeine affect the sucrose feeding response of *Aedes aegypti* (Ignell et al.,

2010). Alkaloids act by activation of a taste neurone called the 'deterrent receptor cell' or *via* inhibition of a receptor cell that responds to phagostimulants (Schoonhoven, 1982).

In recent years, important progress has been made in the understanding of the cellular basis and role of gustatory receptors (Grs) involved in sweet and bitter sensing in *Drosophila* (Dahanukar et al., 2001; Moon et al., 2006; Dahanukar et al., 2007; Jiao et al., 2007; Slone et al., 2007; Weiss et al., 2011). A large family of putative Grs has been identified in the genome of *A. gambiae* (Hill et al., 2002) and the projection patterns of gustatory neurones have been described in detail in the suboesophageal ganglion and the tritocerebrum of this species (Ignell and Hansson, 2005). However, despite the potential importance of deterrent compounds in controlling arthropod-borne diseases, relatively little is known about taste in haematophagous insects. Here we describe feeding experiments with sucrose and its constituents fructose and glucose in *A. gambiae*, and provide insight into sugar perception in this species from electrophysiological recordings performed on a labellar trichoid sensillum containing sucrose- and water-sensitive gustatory receptor cells. We then present the feeding inhibition effects of denatonium benzoate, some quinoline (quinine and quinidine) and isoquinoline (berberine) alkaloids added to sucrose, and account for the inhibitory effect of these compounds on the labellar sucrose- and water-sensitive chemoreceptor cells.

MATERIALS AND METHODS

Anopheles gambiae colony

The *A. gambiae* colony (16cSS strain, derived in 1974 from wild-caught adults originating from Lagos, Nigeria, West Africa) was maintained in a climate chamber [28°C, 80% relative humidity (RH)] under a 12 h:12 h light:dark cycle with 2 h simulated sunrise and sunset. Females fed on a guinea-pig once a week and eggs were recovered on wet filter paper. About 275 larvae were reared in trays in distilled water and fed with pulverized Tetramin fish food. This density provides optimal nutrition for larvae, synchronous eclosion and adults of homogeneous size (Timmermann and Briegel, 1993). Nine to 10 days after oviposition about 800 adult mosquitoes emerged into a rearing cage (350×350×550 mm high) and were provided with 10% (w/v) sucrose and water *ad libitum*.

Test chemicals

D-(–)-fructose, D-(+)-glucose monohydrate, D-(+)-sucrose, berberine chloride, caffeine, quinine anhydrous, denatonium benzoate, NaCl, $CuSO_4$ and $LaCl_3$ were purchased from Sigma-Aldrich (Buchs, Switzerland), (+)-quinidine from Alfa Aesar (Karlsruhe, Germany) and KCl and $CaCl_2$ from Merck (Darmstadt, Germany). Puritiy of all products was ≥98%. Solutions were kept at 10°C, for less than 1 week.

Feeding assays on filter paper

Experiments were performed in a walk-in climate chamber (25°C, 80% RH) during the last 5 h of the scotophase. Four- to six-day-old female *A. gambiae* were individually deprived of water and sugar for 3–5 h in small plastic tubes (15 mm long, 15 mm diameter) closed with two perforated plastic stoppers (diameter 7 mm) with the aperture covered by stainless-steel netting (mesh size: 0.4 mm) to facilitate exchange of air kept in boxes at 95% RH. After this conditioning, the tubes were weighed using a M3 Microbalance (reading precision of ±1 µg; Mettler, Greifensee, Switzerland). For this procedure, the mean standard deviation of weighing five different empty plastic tubes five times was 1 s.d.=2.64 µg. The mosquitoes were then transferred into glass vials (40 mm high, 20 mm diameter) providing adequate space for feeding. Vials were opened and upturned for 30 min on filter paper (55 mm diameter, no. 10 311 807, Whatman Schleicher and Shuell, Dassel, Germany) treated with 600 µl of a test solution in a Petri dish (modified from Arsic and Guerin, 2008). During feeding, a beige plastic container was upturned on the Petri dishes to avoid visual stimuli. Light did not exceed 1 lx under the container. After the feeding experiment, mosquitoes were individually anaesthetized with CO_2 and transferred into the initial plastic tubes and weighed again. The amount of solution ingested was established as the difference in weight before and after feeding. The time between weighings was 60–90 min. Four types of experiments were performed using this bioassay. Firstly, to establish the sucrose level that induces feeding by female *A. gambiae*, solutions of sucrose from 1 to 250 mmol l^{-1} were dissolved in nanopure water. In a second experiment, to measure any phagostimulatory effect of the two hexose sugars that make up sucrose, fructose and glucose were tested individually at 146 and 292 mmol l^{-1}, mixtures of fructose and glucose at 73 mmol l^{-1} each (the same molarity as in 146 mmol l^{-1} sucrose) and at 146 mmol l^{-1} each (i.e. the same mass of glucose and fructose that constitutes 146 mmol l^{-1} sucrose), 146 mmol l^{-1} sucrose (5% w/v) as a positive control and nanopure water as a negative control. To test for feeding inhibition by bitter products, 146 mmol l^{-1} sucrose mixed with solutions of 1 mmol l^{-1} caffeine, berberine, quinine, quinidine, denatonium benzoate and 2 mmol l^{-1} berberine were compared with 146 mmol l^{-1} sucrose as control. In a fourth set of experiments, feeding was quantified for quinine and quinidine at concentrations from 0.01 to 1 mmol l^{-1} in 146 mmol l^{-1} sucrose. Solutions were tested in a random order.

Feeding assays with glass capillaries

Feeding assays were performed under the same environmental conditions as described above from glass capillaries with 4- to 6-day-old female *A. gambiae* at low light (7 lx) to establish if surrounding the mouthparts alone, i.e. excluding tarsal chemosensilla, was sufficient to induce feeding and feeding inhibition by sucrose and quinine presented in sucrose. The mosquitoes were rendered quiescent on ice, and attached by the wings on a glass slide using double-sided adhesive tape 3–7 h before the start of the experiments. During this conditioning period mosquitoes had no access to sucrose or water but were kept in boxes at 95% RH. For these feeding assays, 5 µl glass capillaries (no. 7087 07, Blaubrand, Wertheim, Germany), previously shortened to remove the proximal part without calibration marks, were pulled with an Ealing vertical micropipette puller (no. 50-2012, Harvard Apparatus, Edenbridge, Kent, UK) into two 40 mm segments with a 180 µm internal diameter tip to accommodate the proboscis. Mosquitoes were allowed to feed for 5 min on solutions of 146 mmol l^{-1} sucrose or 146 mmol l^{-1} sucrose + 1 mmol l^{-1} quinine in nanopure water. A blue food colorant (E131) was added to each solution (five drops of 30 mg to 10 ml) to permit visualization of the liquid levels in capillaries. Liquid levels were recorded as images with a scanner (Scanjet 4570c, Hewlett-Packard, Dübendorf, Switzerland) at 600 d.p.i. before and after experiments, and fluid consumption was measured using ImageJ version 1.44 (Rasband, 2011) as described in Sellier et al. (Sellier et al., 2011) using the 1 µl marks on the capillaries as references. To control for possible evaporation within the capillary, the level of liquid in a capillary filled with 146 mmol l^{-1} sucrose solution and manipulated exactly as the test capillaries was likewise measured.

Ultrastructure of trichoid T1 sensilla of the labellar lobes

For transmission electron microscopy, the probosces of *A. gambiae* were fixed in Karnovsky fixative (pH 7.5) overnight at 4°C and rinsed three times in 0.2 mol l^{-1} sodium cacodylate buffer with 4% sucrose. After post-fixation in 1% OsO_4 for 2 h and rinsing in the same buffer, the specimens were stained in block with 2% uranyl acetate (pH 4) in 15% ethanol for 30 min at room temperature, rinsed in 15% ethanol and dehydrated through a graded series of acetone and embedded in Spurr's resin. Ultrathin sections were obtained on a Reichert Ultracut S microtome (Leica, Vienna, Austria) and stained with uranyl acetate and lead citrate. Serial sections were collected every 1 µm from the tip of the first distal trichoid sensillum up to 60 µm of the proboscis from six female and two male *A. gambiae* and were examined in a Philips CM 100 electron microscope (Philips Electron Optics, Eindhoven, The Netherlands) at 60 kV. For scanning electron microscopy, heads of *A. gambiae* were mounted on stubs with Leit-tabs (Plano, Wetzlar, Germany), air-dried for 1 week and coated with a layer of gold in a Sputter coater (Baltec SCD 005; Oerlikon Balzers, Balzers, Lichtenstein). Specimens were examined at 10 kV using a Philips XL 30 scanning electron microscope.

Electrophysiology

Electrophysiological responses of chemoreceptor cells in a trichoid sensillum on the labellum of female *A. gambiae* (Fig. 6A, arrow) were recorded to test stimuli. After being rendered quiescent on ice, mosquitoes were attached by the back on plasticine with two curved tungsten wires, surrounded by moist cotton. After removing the maxillary palps, the mouthparts were fixed with double-sided adhesive tape on a Plexiglas stub. Contact chemosensilla on the mosquito mouthparts were visualized under a microscope (BX51WXIF, Olympus Schweiz, Volketswil, Switzerland) at a magnification of 1000.

Imagoes that had emerged the same morning (0-day-old) were used for electrophysiological recordings when not otherwise specified. Using the method first described by Hodgson et al. (Hodgson et al., 1955), sensilla were stimulated with a recording borosilicate electrode (33 mm long, 14 µm tip diameter) containing the test products diluted in nanopure water. A borosilicate electrode of the same size, mounted on plasticine, filled with haemolymph Ringer solution (Kaissling, 1995), grounded *via* a chlorinated silver wire and inserted into the mosquito head acted as a reference electrode. The recording electrode was connected *via* a chlorinated silver wire to a high impedance 'non-blocking' pre-amplifier (Taste Probe, Syntech, Hilversum, The Netherlands) (Marion-Poll and van der Pers, 1996) mounted on a hydraulic micromanipulator (M0-103, Narishige Scientific Instrument Laboratory, Tokyo, Japan). The electrophysiological signal was amplified ten times with an amplifier in conjunction with a 100–3000 Hz band-pass filter provided in an IDAC-4 intelligent data acquisition controller (Syntech). AC signals were recorded via the IDAC-4 analogue to digital card to a PC equipped with the spike analysis software AutoSpike (version 3.9; Syntech) at a 10 kHz sampling rate. Most concentrations of test products were tested two consecutive times for 2.5–6.5 s at intervals of approximately 2 s. An analysed signal was always the first to be recorded. In cases where a problem occurred during first stimulation (e.g. low signal/noise ratio, insect movement), spikes were quantified from a subsequent stimulation. A delay of 2–5 min was respected between each solution tested

At first, to evaluate the inhibitory effect of salts on the neurophysiological response to water, concentrations from 0.01 to 100 mmol l^{-1} KCl and NaCl, from 0.01 to 1 mmol l^{-1} $CuSO_4$, from 0.01 to 10 mmol l^{-1} $CaCl_2$ and from 0.1 to 1000 µmol l^{-1} $LaCl_3$ in nanopure water were tested in increasing concentration on the sensillum. Each stimulation series started and ended with nanopure water as controls. To check for the presence of a 'sucrose receptor cell', concentrations from 0.1 to 292 mmol l^{-1} sucrose were tested in increasing order in 10 mmol l^{-1} KCl, i.e. at a KCl concentration that inhibits the neurophysiological response to water. Then concentrations from 0 to 100 mmol l^{-1} sucrose in pure water were tested in increasing order to check for the presence of a 'sucrose receptor cell' different from the 'water receptor cell'. This was repeated with 5-day-old mosquitoes, an age that corresponds to the one used in the feeding assays. To study the sugar receptor site(s), fructose and glucose were tested separately at 146 and 292 mmol l^{-1}, mixtures of fructose and glucose (each at 73 mmol l^{-1} and 146 mmol l^{-1}), and compared with 146 and 292 mmol l^{-1} sucrose in 10 mmol l^{-1} KCl as positive controls, and 10 mmol l^{-1} KCl alone as a negative control. The action of bitter products on the responses to water and sucrose by the receptor cells within the trichoid sensillum was investigated by stimulating with concentrations from 0.01 to 1 mmol l^{-1} denatonium benzoate, quinine, berberine or caffeine diluted in nanopure water and in 146 mmol l^{-1} sucrose plus 10 mmol l^{-1} KCl. Each stimulation series started and ended with nanopure water or 146 mmol l^{-1} sucrose in 10 mmol l^{-1} KCl as controls.

Spike discrimination

Spikes were quantified during the 2 s following stimulation. In cases where receptor cells were activated with a delay, spikes were quantified during the 2 s following activation of receptor cells or from a subsequent stimulation showing no delay. All products tested affected receptor cell responses over 2 s. Spikes were discriminated on the basis of amplitudes and shapes using the interactive AutoSpike procedures. Spike amplitudes were sorted using DataView 8.0.0 software (Heitler, 1999) omitting the first 30 ms to avoid the contact artifact. Moreover, to confirm the activation of more than one receptor cell, all recordings were visually inspected for doublets (as presented in Hiroi et al., 2004). Since spike amplitude varies in parallel with frequency in labellar chemosensory cells of *A. gambiae* as in *Drosophila* (Fujishiro et al., 1984), a careful analysis of several recordings was made for different concentrations of products on the same sensillum of the same mosquito (see Results) for the identification of active sensory units.

Statistical analyses

All statistical analyses and graphical representations of data were performed with R 2.11.1 (R Development Core Team, 2010). A logistic model of the type:

$$\text{Meal size} = \text{max}/\left(1+\text{e}\left(\frac{\text{xmid}-\text{conc}}{\text{scal}}\right)\right) \quad (1)$$

was fitted to the meal size data from female *A. gambiae* exposed on filter papers treated with different concentrations of sucrose. In this model max is the maximum response obtained (the horizontal asymptote), xmid is the inflexion point showing the concentration of sucrose at which the meal size is theoretically half of a complete meal (ED_{50}), conc is the sucrose concentration and scal is a numeric scale parameter that is equal to max/(4×slope) at the inflection point of the model.

To establish differences between sugar phagostimulatory effects, the meal sizes of female *A. gambiae* exposed to filter papers treated with sucrose, fructose, glucose and the two mixtures of fructose and glucose were analysed using one-way ANOVA on the ranked values

(discrete data; normal distribution, Shapiro–Wilk test, $P>0.05$; homogeneous variance, Bartlett's test, $P>0.05$). The model was simplified by aggregating non-significant factor levels in a stepwise *a posteriori* procedure. Similar analyses were performed on the meal sizes of female *A. gambiae* exposed to filter papers treated with 146 mmol l^{-1} sucrose and 146 mmol l^{-1} sucrose with bitter products added. To analyse feeding inhibition by quinine and quinidine as a function of concentration and to check for possible interactions between products and concentrations, an ANCOVA based on a linear model was made on the ranked meal sizes (continuous data; normal distribution, Shapiro–Wilk test, $P>0.05$; homogeneous variance, Bartlett's test, $P>0.05$) of female *A. gambiae* exposed to different concentrations of quinine and quinidine added to 146 mmol l^{-1} sucrose. In the feeding assay with glass capillaries, meal sizes (normal distribution, Shapiro–Wilk test, $P>0.05$; homogeneous variance, Bartlett's test, $P>0.05$) of females exposed to 146 mmol l^{-1} sucrose and 146 mmol l^{-1} sucrose with 1 mmol l^{-1} quinine added were compared using the *t*-test.

To study the inhibition of the neurophysiological response to water by salts, the mean of the total spike numbers per 2 s (MTNoS, normal distribution, Shapiro–Wilk test, $P>0.05$, but with inhomogeneous variance, Bartlett's test, $P<0.05$) generated by the water receptor cell for the different concentrations tested were compared with the first water control using Welch's *t*-test. A two-parameter asymptotic exponential model of the type:

$$N \text{ spikes} = \max\,(1 - e^{(-c \times \text{conc})}), \quad (2)$$

was found to be the most suitable to account for the number of action potentials generated by the sucrose-sensitive receptor cell per 2 s to increasing concentrations of sucrose in 10 mmol l^{-1} KCl and water. In this model, max is the maximum response obtained (the horizontal asymptote), *c* is the rate constant and conc is the sucrose concentration. The spike numbers recorded per 2 s for the different fructose and glucose mixtures and sucrose solutions in 10 mmol l^{-1} KCl tested (discrete data; normal distribution, Shapiro–Wilk test, $P>0.05$; homogeneous variance, Bartlett's test, $P>0.05$) were compared by a one-way ANOVA. To analyse sensory inhibition by bitter products and to check for interactions between products and concentrations on sensory cell responses, an ANCOVA based on a linear model (continuous data; normal distribution, Shapiro–Wilk test, $P>0.05$; homogeneous variance, Bartlett's test, $P>0.05$) was applied to the number of action potentials per 2 s as a function of the $\log_{10}$ of the concentration of bitter products added to nanopure water or to 146 mmol l^{-1} sucrose in 10 mmol l^{-1} KCl. The bracketing controls were excluded from the analysis to comply with the requirement of using continuous variables in the linear model employed. A 95% confidence interval level was used for all analyses.

RESULTS

Feeding assays on filter paper

Feeding responses to increasing concentrations of sucrose

The calculated model indicates that starved 4- to 6-day-old female *A. gambiae* start to feed at concentrations between 50 and 75 mmol l^{-1} with meal size estimated at 0.045 mg at 50 mmol l^{-1}, and 0.088 mg at 75 mmol l^{-1} sucrose (Fig. 1). At 250 mmol l^{-1}, mosquitoes engorge 0.376 mg of sucrose solution, close to the asymptote of the model (0.378 mg), an amount corresponding to 30% of their weight prior to feeding. ED_{50} is calculated from the model to occur at 111.09 mmol l^{-1} sucrose with a meal size of 0.19 mg. At 1 and 10 mmol l^{-1} sucrose, mosquitoes imbibe almost nothing (0.01 mg), comparable to the water control [median meal size (MMS), –0.007 mg]. Zero-day-old females can discriminate between water and 292 mmol l^{-1} sucrose like the 4- to 6-day-old females in our assay (data not presented).

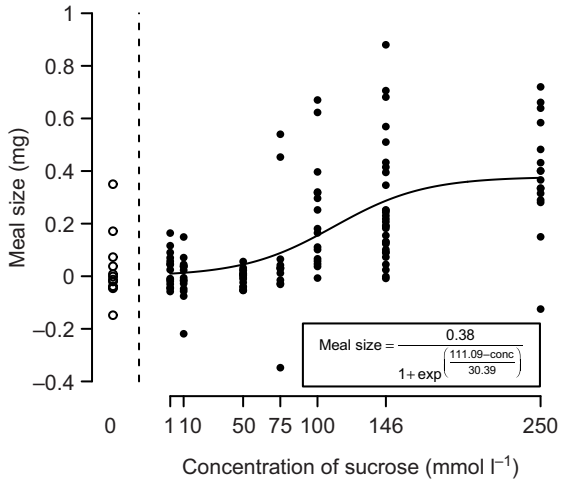

Fig. 1. Logistic model of meal size in female *A. gambiae* following 30 min exposure on filter paper treated with different concentrations of sucrose (conc in the equation). At concentrations of sucrose higher than 250 mmol l^{-1}, meal sizes tend to 0.38 mg and the inflexion point of the model is situated at 111.09 mmol l^{-1}. Water (without sucrose, open circles on left) was not included in the model. Between 12 and 30 mosquitoes were tested per concentration.

Feeding responses to fructose and glucose

Following ANOVA on the ranked meal sizes on fructose, glucose, their binary mixtures and sucrose followed by a stepwise aggregation of non-significant factor levels, three significant contrasts are retained in the final model ($F_{2,159}=35.09$, $P<0.001$, Fig. 2). Meal sizes on water (MMS, –0.021 mg), on 146 or 292 mmol l^{-1} glucose (MMS, –0.0025 and 0.027 mg, respectively) and on 73 mmol l^{-1} glucose plus fructose (MMS, –0.0135 mg) are low and not significantly different. While there are no significant differences between 146 and 292 mmol l^{-1} fructose (MMS, 0.046 and 0.0505, respectively), fructose appears to have a mild phagostimulatory effect on female *A. gambiae* as meal sizes are significantly higher than on water, on 146 and 292 mmol l^{-1} glucose or on 73 mmol l^{-1} glucose plus 73 mmol l^{-1} fructose ($P=0.002$), but lower than on 146 mmol l^{-1} glucose plus 146 mmol l^{-1} fructose and on 146 mmol l^{-1} sucrose ($P<0.001$). The mixture of 146 mmol l^{-1} glucose plus 146 mmol l^{-1} fructose (MMS, 0.133 mg) and 146 mmol l^{-1} sucrose alone (MMS, 0.137 mg) have a similarly strong phagostimulatory effect on starved female *A. gambiae*.

Inhibitory effect of bitter products on the sucrose-feeding response

Except for caffeine, all bitter products tested significantly reduced feeding in *A. gambiae* when added to a solution of 146 mmol l^{-1} sucrose (Fig. 3). Following ANOVA on the ranked meal sizes followed by a stepwise aggregation of non-significant factor levels, three significant contrasts were retained in the final model ($F_{2,156}=101.4$, $P<0.001$). When 1 mmol l^{-1} caffeine is added to 146 mmol l^{-1} sucrose the meal size of starved female *A. gambiae* is not significantly reduced (MMS, 0.184 mg) compared with 146 mmol l^{-1} sucrose alone (MMS, 0.204 mg). Although no significant difference in meal size was found between 1 mmol l^{-1} berberine (MMS, 0.121 mg) and 2 mmol l^{-1} berberine (MMS,

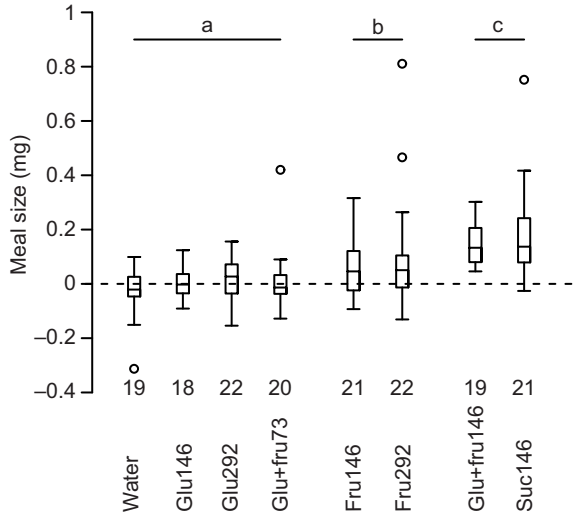

Fig. 2. Meal size of female *A. gambiae* after 30 min exposure on filter paper treated with either water, 146 mmol l^{-1} glucose (Glu146), 292 mmol l^{-1} glucose (Glu292), 73 mmol l^{-1} glucose plus 73 mmol l^{-1} fructose (Glu+fru73), 146 mmol l^{-1} fructose (Fru146), 292 mmol l^{-1} fructose (Fru292), 146 mmol l^{-1} glucose plus 146 mmol l^{-1} fructose (Glu+fru146), and 146 mmol l^{-1} sucrose (Suc146). Groups of products and their mixtures with different letters have a significantly different effect on meal size with a 95% confidence level (ANOVA on the ranked meal sizes; non-significant factor levels were aggregated using a stepwise *a posteriori* procedure). The number of mosquitoes tested is indicated under each box plot. Box plots represent the median (black bars), the 25–75% interquartile range (IQR) intervals (boxes), the lowest and the highest data points still within 1.5 of the IQR (whiskers) and outliers (circles).

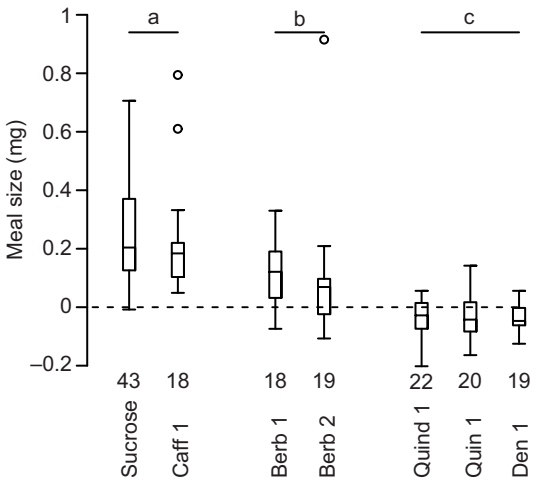

Fig. 3. Meal size of female *A. gambiae* after 30 min exposure on filter paper treated with 146 mmol l^{-1} sucrose and 146 mmol l^{-1} sucrose with 1 mmol l^{-1} caffeine (Caff), 1 mmol l^{-1} berberine (Berb 1), 1 mmol l^{-1} quinidine (Quind), 1 mmol l^{-1} quinine (Quin), 1 mmol l^{-1} denatonium benzoate (Den) or 2 mmol l^{-1} berberine (Berb 2) added. Groups of products with different letters have a significantly different effect on the meal size with a 95% confidence level (ANOVA on the ranked meal sizes; non-significant factor levels were aggregated using a stepwise *a posteriori* procedure). The number of mosquitoes tested is indicated under each box plot. For explanation of box plots, see legend to Fig. 1.

0.069 mg), both concentrations of berberine have an intermediate deterrent effect, with meal sizes significantly lower than 1 mmol l^{-1} caffeine in sucrose and 146 mmol l^{-1} sucrose alone (P<0.001), but significantly higher than 1 mmol l^{-1} quinidine, quinine and denatonium benzoate (MMS, –0.028, –0.0425 and –0.047 mg, respectively, P<0.001) all of which almost completely suppress feeding on 146 mmol l^{-1} sucrose. Although the deterrent effect of both quinidine and quinine clearly depends on concentration (Fig. 4), no significant interactions and no significant differences in the intercepts of the linear equations describing the responses occur between these two products ($F_{3,115}$=40.85, R^2=0.5, P<0.001). Meal sizes on water are not significantly different from meal sizes of mosquitoes exposed to 5% sucrose mixed with 1 mmol l^{-1} quinine, quinidine and denatonium benzoate (ANOVA on the ranked values, P=0.296), allowing us to conclude that mosquitoes do not significantly lose more liquid by diuresis or regurgitation in the presence of the bitter compounds tested.

Feeding assays with glass capillaries

Five minutes are sufficient for starved female *A. gambiae* to engorge on 146 mmol l^{-1} sucrose from glass capillaries (mean ingested volume 0.34 µl; Fig. 5), a level approaching that recorded on filter paper. Quinine at 1 mmol l^{-1} significantly reduced the volume ingested by female *A. gambiae* when added to 146 mmol l^{-1} sucrose (mean ingested volume: 0.15 µl, t=3.13, d.f.=28, P=0.004; Fig. 5). The mean level of evaporated liquid from capillaries was negligible (0.029 µl). As tarsal contact chemosensilla were not in contact with the solution, this indicates that contact chemosensilla either on the mouthparts or in the cibarium are involved in the perception of sucrose and alkaloids. For this reason the electrophysiological recordings described below focused on mouthpart sensilla.

Ultrastructure of trichoid T1 sensilla of the labellar lobes

Long trichoid sensilla of between 30 and 35 µm occur on the external surface of the labellar lobes of both male and female *A. gambiae* (Fig. 6A). These are typical gustatory sensilla with a terminal pore (Fig. 6B) and two lymphatic spaces divided by a cuticular wall (Fig. 6C). The inner receptor lymph cavity (Fig. 6C) contains four chemosensory dendrites and the outer lymph cavity contains an electron-dense fluid (Fig. 6C). In all, five neurones innervate each sensillum (Fig. 6D). One dendrite terminates in a tubular body (Fig. 6D) at the base of the hair, while the other four extend into the inner lymph cavity to the tip of the hair close to the terminal pore (Fig. 6C). At the base, the five outer dendritic segments are surrounded by a sheath, secreted by the thecogen cell, which ends below the cuticle of the socket (Fig. 6D). Further down, the three enveloping cells (thecogen, trichogen and tormogen) encase the five ciliary roots (with 9×2+0 microtubules) and the inner dendritic parts of the sensory cells (Fig. 6E). The morphology of the trichoid sensillum described here on the labellum of female *A. gambiae* appears to be very similar to the long labellar trichoid type 1 sensilla (T1) described by Pappas and Larsen (Pappas and Larsen, 1976) in *Culiseta inornata* (Williston) and those described by Owen (Owen, 1971) and Hill and Smith (Hill and Smith, 1999) in *Anopheles atroparvus* (Van Thiel) and *A. aegypti*, respectively. About 10 T1 sensilla occur on the dorsal and ventral sides of each labellar lobe of female and male *A. gambiae* and no differences have been found between the sexes in either the placement or morphology of T1 sensilla in this species. One dendrite appears to be larger than the other three (measured midway along four different T1 sensilla of three females; Fig. 6C).

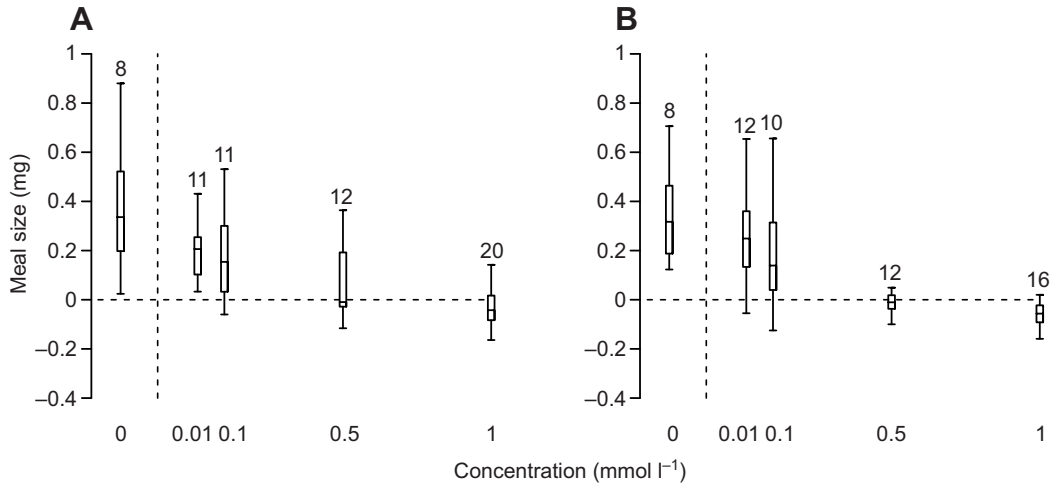

Fig. 4. Concentration–response effects on meal size in female *A. gambiae* following 30 min exposure to filter paper treated with different concentrations of quinine (A) and quinidine (B) in 146 mmol l^{-1} sucrose. The number of mosquitoes tested is indicated above each box plot. Box plots on the left of A and B represent meal sizes on 146 mmol l^{-1} sucrose without an alkaloid. For explanation of box plots, see legend to Fig. 1.

Electrophysiology

Electrophysiological responses to increasing concentrations of salts

When the trichoid sensillum (indicated in Fig. 6A) is stimulated with nanopure water, one receptor cell is activated at a mean frequency of 64.12±1.97 spikes per 2 s. However, this receptor cell is inhibited by increasing concentrations of salts, with $CuSO_4$ and $LaCl_3$ being the most inhibitory (Figs 7, 8). Inhibition is already significant in comparison with the water control at 0.5 mmol l^{-1} $CuSO_4$ (P<0.001) where the mean total number of spikes per 2 s (MTNoS) is reduced by 60% (Fig. 7D). $LaCl_3$ significantly inhibits the water response at 1 mmol l^{-1} (P<0.001) with a drop of 92% in the MTNoS recorded compared with the first water control (Fig. 7F). The inhibitory effects of 1 mmol l^{-1} KCl, NaCl or $CaCl_2$ are lower, with a drop in the MTNoS of 40, 50 and 59%, respectively, but significantly different from the water control (P=0.002, 0.002 and 0.02, respectively; Figs 7, 8). The increase (six spikes) in the MTNoS observed between 0 and 0.1 mmol l^{-1} $CaCl_2$ is not significant (P=0.525). Between 10 and 100 mmol l^{-1} KCl and NaCl the MTNoS increases slightly (10 and 15 per 2 s, respectively; Fig. 7) due to the appearance of predominantly negative-going spikes at these levels of salts (Fig. 7C; Fig. 8A,B). Such spikes could arise as an artifact at high salt levels or represent activation of another cell. All spikes quantified in these experiments could be readily discriminated from the spikes of the mechanoreceptor cell, which are readily visualized at a lower amplitude when the sensillum is pulled proximally (Fig. 8C). In 50% of the mosquitoes tested, the water receptor cell was activated after a delay of up to 2 s following electrode contact with the sensillum tip (Fig. 9A). In such cases a second stimulation was generally made not more than 2 s later and the receptor cell then responded immediately in a phasic–tonic manner (Fig. 9B).

Electrophysiological responses to increasing concentration of sucrose in 10 mmol l^{-1} KCl

The electrophysiological response to increasing concentrations of sucrose was firstly characterized in 10 mmol l^{-1} KCl in order to inhibit the water receptor cell. The action potential frequency is modulated by sucrose concentrations between 0.1 and 292 mmol l^{-1} following the model:

$$N \text{ spikes} = 57.35\,(1 - e^{(-0.17\times\text{conc})}). \quad (3)$$

The mean number of spikes per 2 s (MNS) of the sucrose receptor cell increases strongly from 0.1 mmol l^{-1} (MNS 0.75±0.75) to 50 mmol l^{-1} sucrose (MNS 62.33±3.73) in 10 mmol l^{-1} KCl (Fig. 10; Fig. 11A–C). The frequency of action potentials emitted by the sucrose-sensitive neurone tends to an asymptote (57.35 spikes per 2 s) from 50 mmol l^{-1} in 10 mmol l^{-1} KCl. Half of the maximum frequency (ED_{50}) is estimated at 4.07 mmol l^{-1} sucrose with 28.67 spikes per 2 s. The amplitude of spikes generated by the sucrose receptor cell increases slightly from 3.0±0.2 to 3.8±0.4 mV between 1 and 50 mmol l^{-1}, followed by a drop to 2.0±0.2 mV at 292 mmol l^{-1}, possibly due to the increased resistance caused by the high sucrose concentration in the electrode (Fig. 11D; supplementary material Fig. S1). At 0 mmol l^{-1} sucrose in 10 mmol l^{-1} KCl, no spikes are generated from the sucrose receptor cell. Only spikes of smaller amplitude with a predominantly negative-going shape are observed (Fig. 11A). These spikes could be generated by the water neurone, not totally inhibited by 10 mmol l^{-1} KCl, or by a third sensory neurone sensitive to salt (see above). In Fig. 11B the two types of spike amplitudes are observed during stimulation with 2.5 mmol l^{-1} sucrose in 10 mmol l^{-1} KCl. The smallest spike amplitude class is inhibited as the concentration of sucrose increases and drops to a MNS of 3 at 50 mmol l^{-1}. The same delay, as observed for the activation of the water neurone, was observed for the activation of the sucrose receptor cell.

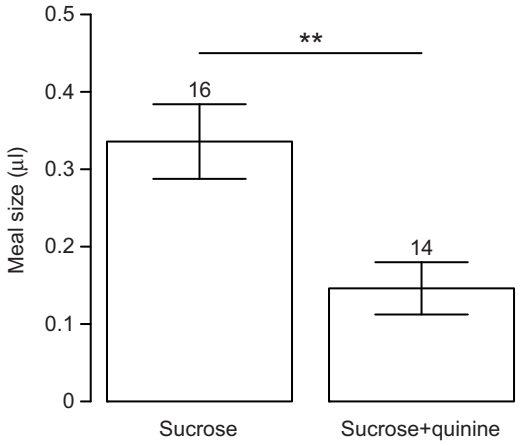

Fig. 5. Mean ± s.e.m. meal size by female *A. gambiae* with their mouthparts inserted for 5 min into glass capillaries containing 146 mmol l^{-1} sucrose and 146 mmol l^{-1} sucrose + 1 mmol l^{-1} quinine. The number of mosquitoes tested is indicated above each bar plot. Mosquitoes fed significantly less from the capillaries with quinine added; t-test, **P<0.01.

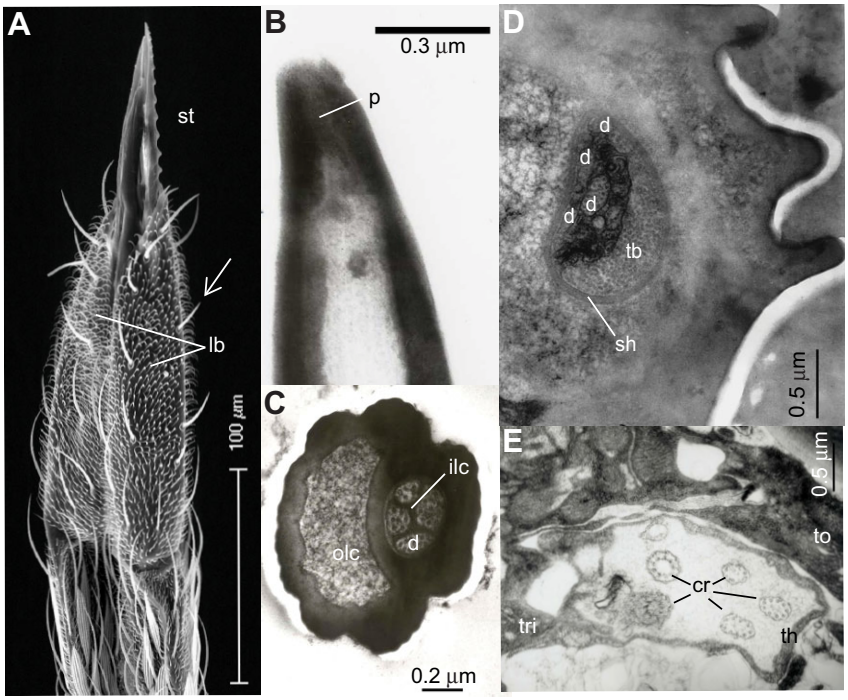

Fig. 6. Ultrastructure of trichoid type 1 sensilla on the proboscis of female *A. gambiae*. (A) Scanning electron micrograph of the extremity of the proboscis (dorsal view) showing the positions of several trichoid T1 sensilla. The arrow indicates the sensillum from which electrophysiological recordings were made in this study. Transmission electron micrographs of a longitudinal section at the tip of a trichoid T1 sensillum (B) and transversal sections at the middle (C), base (D) and at the level of the ciliary roots of a trichoid T1 sensillum (E). cr, ciliary roots; d, chemosensory dendrites; ilc, inner lymph cavity housing the four chemosensory dendrites; lb, labellar lobes; olc, outer lymph cavity; p, terminal pore; sh, sheath; st, stylets; tb, tubular body; th, thecogen cell; to, tormogen cell; tri, trichogen cell.

Discrimination between the water cell and sucrose receptor cell

To determine whether different cells are activated by water and sucrose, increasing concentrations of sucrose between 0.01 and 100 mmol l^{-1} were tested in nanopure water. The water receptor cell fires at a MNS of 61.92±5.35 when the sensillum of 0-day-old mosquitoes is stimulated by pure water. As the sucrose concentration increases, a second spike amplitude category appears from 0.1 mmol l^{-1} with increasing amplitude in parallel with frequency (Fig. 12A,C). At 10 mmol l^{-1} sucrose in water, the amplitude distribution is bimodal in 100% of the recordings (shown in the histograms on the right-hand side of each electrophysiological recording; Fig. 13). This confirms the presence of two activated sensory units. Two electrophysiological traces were removed from the analysis as two sensory units, whose presence was confirmed by the presence of doublets, could not be discriminated on the basis of amplitude, something never observed when sucrose was diluted

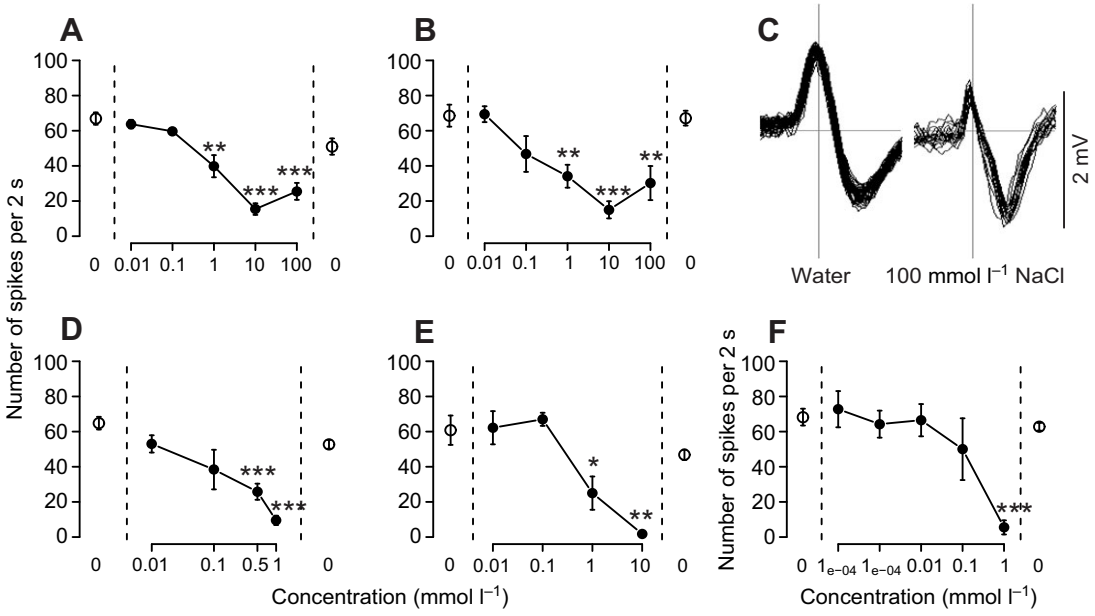

Fig. 7. Mean ± s.e.m. total number of spikes generated by sensory cells within trichoid T1 sensilla on the labellum of female *A. gambiae* during stimulation by water as a function of the $\log_{10}$ concentrations of (A) KCl (12 mosquitoes tested), (B) NaCl (10 mosquitoes tested), (D) $CuSO_4$ (five mosquitoes tested), (E) $CaCl_2$ (five mosquitoes tested) and (F) $LaCl_3$ (four mosquitoes tested). (C) Superimposition of action potentials recorded during stimulation with water (left) and with 100 mmol l^{-1} NaCl (right) displaying differences in shapes and amplitudes of the generated action potentials. Asterisks indicate numbers of spikes generated by the water neurone that are significantly lower than for the initial water controls (presented on the left of each graph); Welch's *t*-test, **P*<0.05, ***P*<0.01, ****P*<0.001.

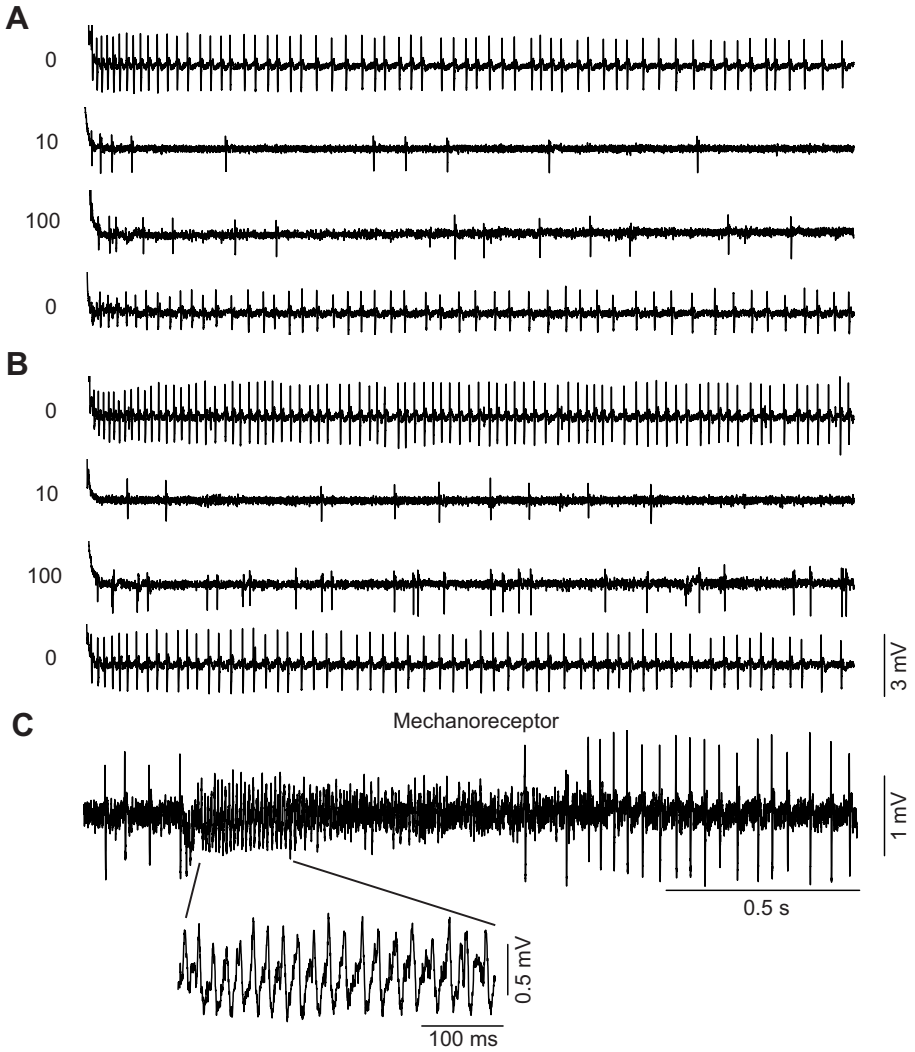

Fig. 8. Representative 2 s electrophysiological responses in trichoid T1 labellar sensilla stimulated with 10 and 100 mmol l^{-1} KCl (A) and NaCl (B). The recording series presented for each salt starts and ends with water (top and bottom traces in each case). (C) Response to water accompanied by mechanical stimulation: when the sensillum is pulled proximally with an electrode filled with pure water the water receptor cell is silenced and the mechanoreceptor is activated. The water-sensitive neurone fires again when the initial position of the sensillum is restored.

in 10 mmol l^{-1} KCl. Attributing spike amplitudes to the sucrose or water receptor cell is based on the fact that spike amplitudes generated by these cells vary in parallel with frequency (Fujishiro et al., 1984). This observation on labellar chemoreceptor cells of *Drosophila* is confirmed here for the labellar sucrose and water cells of *A. gambiae* (Fig. 12A,C). Thus at the sucrose concentration higher than the one where the two spike amplitudes are most similar, the attributions assigned to the two sensory cells is switched for purposes of counting, i.e. the spike amplitude previously assigned to the sucrose cell becomes the water cell and the spike amplitude previously assigned to the water cell becomes the sucrose cell. The concentration at which the mean amplitude is most similar is 5 mmol l^{-1} (Fig. 12A) but this can vary between 1 and 50 mmol l^{-1} depending on the mosquito tested.

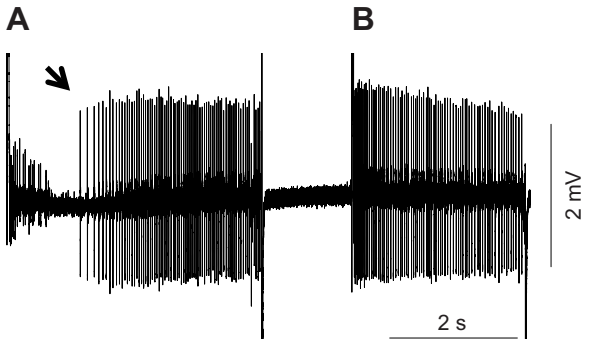

Fig. 9. Two electrophysiological responses to water recorded from a T1 sensillum on the labellum of female *A. gambiae*. (A) On first contact the water receptor cell is typically activated with a delay (arrow at 1.127 s in this example). The mechanoreceptor response is visible during the first 620 ms of this recording, but this is not systematically the case. (B) When a second recording is performed with the same electrode within 1.5 s from the end of the first stimulation, no delay in the response of the water cell is observed. The same phenomenon is observed for the sucrose receptor cell.

Applying this methodology to each recording independently, we calculated the following model to describe the increasing frequency of the sugar receptor cell to increasing concentrations of sucrose dissolved in water:

$$N \text{ spikes} = 53.46\,(1 - e^{(-0.15\times\text{conc})}). \quad (4)$$

An almost identical model was calculated (above) from the data describing the response of the sugar cell to sucrose presented in 10 mmol l^{-1} KCl, i.e. where the water cell is inhibited. This confirms the validity of our spike discrimination method. The number of action potentials per 2 s of the sucrose receptor cell increases strongly from 0.1 mmol l^{-1} (MNS 1.83±0.84) to 25 mmol l^{-1} sucrose (MNS 56.08±5.15) (Fig. 12C). The frequency of the action potentials emitted by the sucrose-sensitive neurone tends to the asymptote (53.46 spikes per 2 s) from 25 mmol l^{-1}. Half of the maximum frequency (ED_{50}) is estimated at 4.62 mmol l^{-1} sucrose in water with

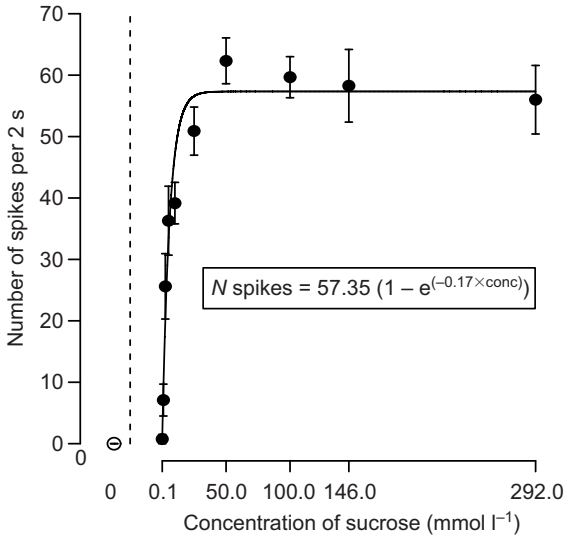

Fig. 10. Asymptotic model of the number of spikes (mean ± s.e.m.) per 2 s emitted by the sucrose-sensitive receptor cell in trichoid T1 sensilla on the labellum of female *A. gambiae* to stimulation with 0, 0.1, 1, 2.5, 5, 10, 25, 50, 100, 146 and 292 mmol l^{-1} sucrose (conc in the equation) presented at increasing concentrations in 10 mmol l^{-1} KCl. The response to 10 mmol l^{-1} KCl alone (open circle on left) is not taken into account in the model (four to 13 mosquitoes tested per concentration).

26.73 spikes per 2 s, close to the ED_{50} calculated for sucrose in 10 mmol l^{-1} KCl at 4.07 mmol l^{-1}. Increasing osmolarity of the stimulating solution by sucrose induces inhibition of the water receptor cell, which generates a MNS of 12.23±4.33 at 100 mmol l^{-1} sucrose (Fig. 12C). No negatively going spikes were recorded with these treatments. Sucrose and water receptor cells were also identified in five 5-day-old female *A. gambiae* by testing increasing concentrations of sucrose between 0.01 and 100 mmol l^{-1} in nanopure water (Fig. 12B,D). The model representing spike frequency of the sucrose receptor cell for the 5-day-old mosquitoes as a function of concentration is:

$$N \text{ spikes} = 96.02\,(1 - e^{(-0.35\times\text{conc})}). \quad (5)$$

This indicates that the asymptote for spikes generated by the sucrose cell of 5-day-old mosquitoes is some two times higher than that of 0-day-old mosquitoes (supplementary material Fig. S2). The model already tends towards the asymptote between 10 mmol l^{-1} (93.05 spikes per 2 s) and 25 mmol l^{-1} (96 spikes per 2 s), and the ED_{50} is situated at 1.99 mmol l^{-1}, more than two times lower than that for 0-day-old mosquitoes.

Electrophysiological responses to fructose, glucose and sucrose

The sucrose receptor cell is hardly activated when the labellar trichoid sensillum is stimulated with either 146 or 292 mmol l^{-1} glucose (MNS 1.2±1.2 and 1.4±1.4, respectively) with 146 or 292 mmol l^{-1} fructose (MNS 0±0 and 4.20±1.91, respectively) in 10 mmol l^{-1} KCl or the control (10 mmol l^{-1} KCl alone; MNS 0±0, P=0.11; Fig. 14). Nevertheless, a mixture of 73 mmol l^{-1} glucose plus 73 mmol l^{-1} fructose (MNS 37.00±3.94), 146 mmol l^{-1} glucose plus 146 mmol l^{-1} fructose (MNS 36.80±5.29), 146 mmol l^{-1} sucrose (MNS 65.20±10.33) and 292 mmol l^{-1} sucrose (MNS 42.25±11.09) activates the sucrose receptor cell (Fig. 14A,B). The number of action potentials recorded per 2 s tend only to be different ($F_{3,15}$=3.04, P=0.06) for 146 mmol l^{-1} sucrose compared with 73 mmol l^{-1} fructose plus 73 mmol l^{-1} glucose, 146 mmol l^{-1} fructose plus 146 mmol l^{-1} glucose and 292 mmol l^{-1} sucrose in 10 mmol l^{-1} KCl (Fig. 14).

Inhibitory effect of bitter products on the sucrose and water receptor cells

Except for caffeine, all the bitter products tested inhibit both the sucrose and water cells at concentrations between 0.01 and 1 mmol l^{-1} (Figs 15, 16), and inhibition is accompanied by a decrease

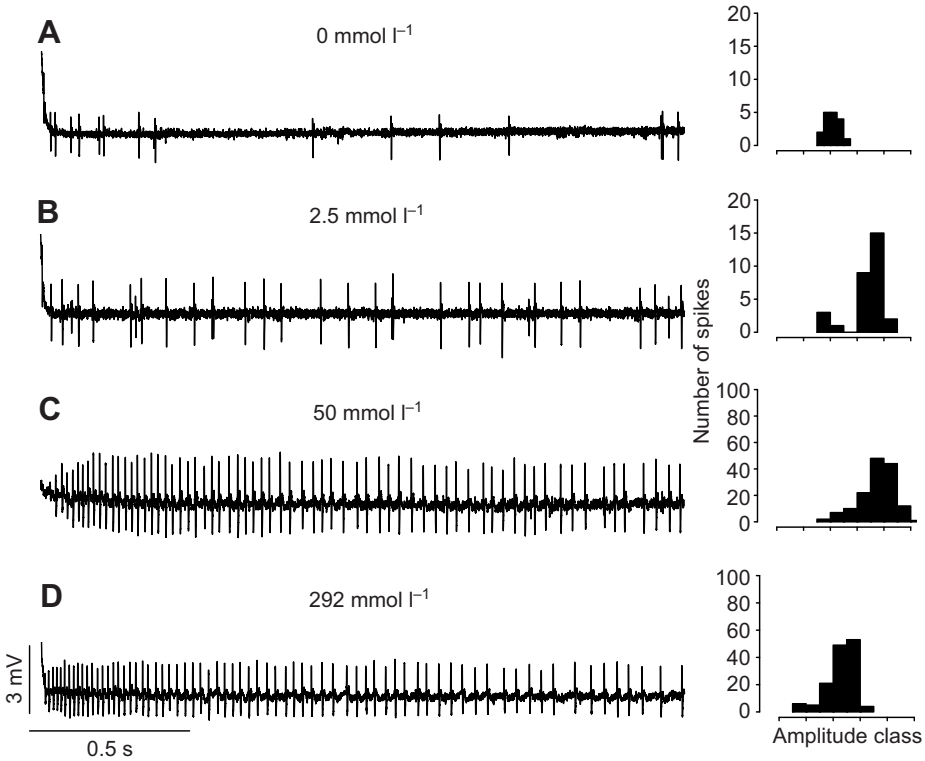

Fig. 11. Examples of recordings from a trichoid T1 sensillum to increasing concentrations of sucrose in 10 mmol l^{-1} KCl and the corresponding spike amplitude histograms (on right; amplitude classes assigned by AutoSpike). With 10 mmol l^{-1} KCl only, spikes with a smaller amplitude than the sucrose cell with a predominantly negative aspect are generated (A). At 2.5 mmol l^{-1}, the sucrose cell is already activated and characterized by spike amplitude greater than those generated in response to 10 mmol l^{-1} KCl alone (as indicated by the bimodal distribution of amplitude classes). At 50 mmol l^{-1} (C) and 292 mmol l^{-1} (D) the sucrose cell dominates and the cell generating small spikes is silenced. Note the decrease in amplitude of the sucrose cell spikes at 292 mmol l^{-1} sucrose.

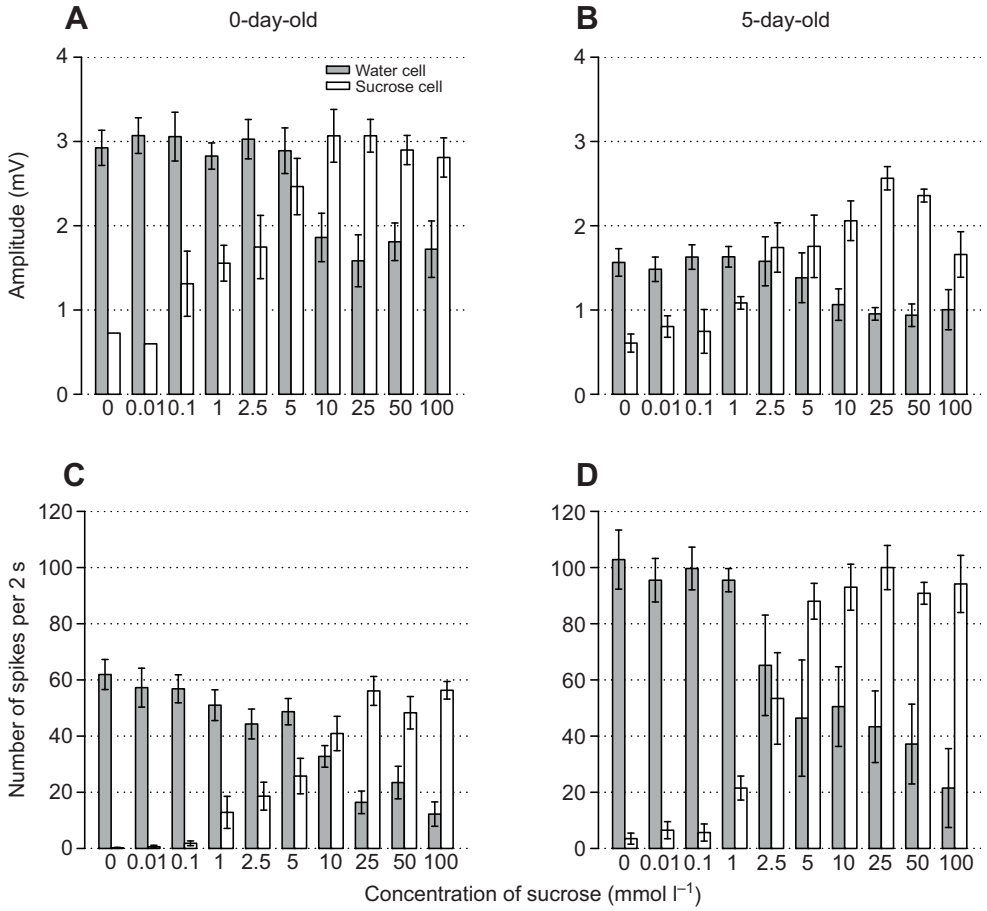

Fig. 12. Mean ± s.e.m. of spike amplitudes recorded from T1 sensilla on the labellum of female *A. gambiae* in response to stimulation with 0, 0.01, 0.1, 1, 2.5, 5, 10, 25, 50 and 100 mmol l^{-1} sucrose in pure water, tested at increasing concentrations on 0-day-old (A) and 5-day-old mosquitoes (B). Number of spikes per 2 s (mean ± s.e.m.) for the corresponding recordings for 0-day-old (C) and 5-day-old mosquitoes (D). The sucrose cell of the 0-day-old mosquitoes is activated in the same way as with sucrose in 10 mmol l^{-1} KCl (see Fig. 10); 5–13 mosquitoes tested per concentration.

in spike amplitude (supplementary material Fig. S3A,B). A linear model fitted to the number of spikes per 2 s as a function of the $\log_{10}$ of concentration of the bitter products proved significant for all inhibitory effects ($F_{9,186}$= 37.34, P<0.001, R^2=0.63; Fig. 15). At the highest concentration tested (1 mmol l^{-1}), one which corresponds to the intercept in the model, nine action potentials less are generated for all bitter products tested in water than in 146 mmol l^{-1} sucrose (P=0.003). The slopes fitted by the model are also significantly steeper for water than for sucrose (P=0.03), suggesting that bitter products are more efficient at inhibiting the water than the sucrose receptor cell. Denatonium benzoate at 1 mmol l^{-1} is significantly more efficient at inhibiting both the sucrose and the water receptor cells (MNS 6.62±2.18; Fig. 15A,E) than quinine (MNS 13.71±3.24, P=0.02; Fig. 15B,F), berberine (MNS 16.42±4.22, P=0.02; Fig. 15C,G) and caffeine (MNS 51±4.94, P<0.001; Fig. 15D,H). The slope of the model is significantly less steep for berberine than for denatonium benzoate (P=0.006) due to the high number of action potentials recorded at 1 mmol l^{-1} berberine in sucrose or water and the greater ability of berberine to inhibit both the sucrose and the water receptor cells at 0.01 mmol l^{-1} in sucrose (Fig. 15C,G). Caffeine has no effect on the electrophysiological response of the sucrose and the water receptor cells with a slope not significantly different from 0 in water or sucrose (P=0.39; Fig. 15D,H). Both the phasic and tonic part of the response of the sucrose and water neurones is affected by bitter products, as shown in supplementary material Fig. S4A,B for inhibition by quinine. No receptor cell appears to be activated by the concentration range of the bitter products tested in the labellar sensillum on which the present study focuses.

DISCUSSION

Sensing and feeding on sucrose by *A. gambiae*

In this study sucrose proved a strong phagostimulant for starved female *A. gambiae*. Mosquitoes start to feed at concentrations between 50 and 75 mmol l^{-1} sucrose in solution. ED_{50} is reached at around 111 mmol l^{-1}, the inflexion point of the feeding model. Small changes in the sucrose concentration at this dose where the slope of the response is at its steepest (0.625%) have the highest effect on meal size. However, the amount of ingested solution reaches a maximum between 146 and 250 mmol l^{-1} sucrose. Since females engorge on a 146 mmol l^{-1} sucrose solution from a glass capillary in a comparable manner, this permits us to conclude that the presence of receptor cells on the mouthparts (eventually within the cibarium) sensitive to sucrose are sufficient to elicit feeding. The glass capillary feeding experiments indicate that tarsal receptor cells are apparently not necessary to induce feeding on sucrose. It should be remembered that *A. gambiae* females feed on NaCl solutions only when heated as in blood and the meal is directed to the mid-gut, whereas a sucrose solution needs to be at room temperature and is directed to the crop (Arsic and Guerin, 2008).

Our electrophysiological study focuses on two labellar receptor cells: one is activated by sucrose in a concentration-dependent manner, and a second is activated by water and inhibited by sucrose and salts. The electrophysiological response of the sucrose receptor cell to sucrose diluted in water starts well below the levels of sugar necessary to induce engorgement. For both 0- and 5-day-old mosquitoes, the slopes of the two electrophysiological models are steepest at 801 and 3326%, respectively, at 0.01 mmol l^{-1} sucrose in water, decreasing to 179 and 103%, respectively, at 10 mmol l^{-1},

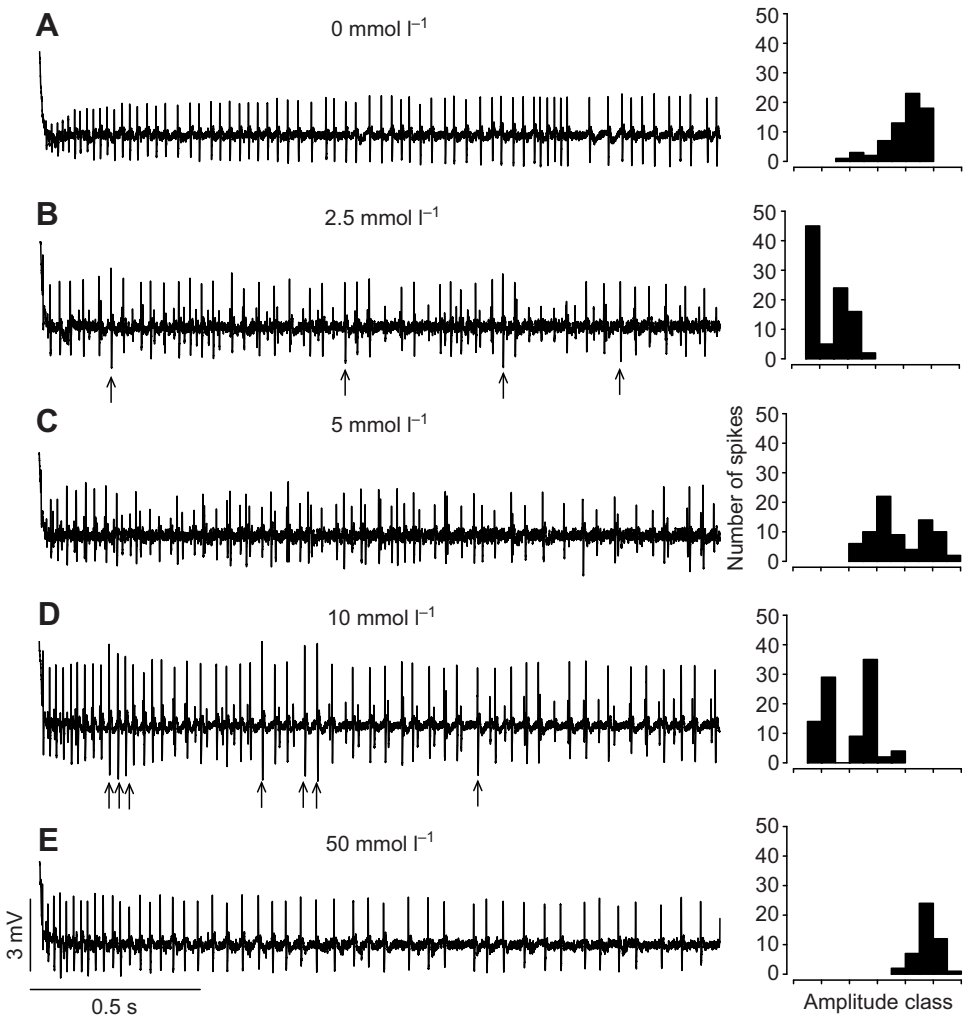

Fig. 13. Examples of recordings from a T1 trichoid sensillum to increasing concentrations of sucrose in pure water and their corresponding spike amplitude histograms (on right; amplitude classes assigned by AutoSpike). At 0 mmol l^{-1} sucrose, only the water cell is active (A). At 2.5 mmol l^{-1} (B) the sucrose cell is already active with a spike amplitude lower than that generated by the water cell (bimodal distribution of amplitude classes). At 5 mmol l^{-1} (C), although the amplitude of the spikes of the sucrose cell is still lower than that generated by the water cell, the amplitudes of both receptor cells move closer. At 10 mmol l^{-1} (D) the sucrose cell is more active with a spike amplitude higher than that generated by the water cell. At 50 mmol l^{-1} (E) the water cell is silenced on this recording and only spikes from the sucrose receptor cell are visible. Superimpositions of spikes are marked with arrows on recordings B and D.

and plateaus from approximately 25 mmol l^{-1} for both ages. This shows that *A. gambiae* is most sensitive to small increments in sucrose concentration up to 10 mmol l^{-1}. An ED_{50} is recorded at 1.99 mmol l^{-1} sucrose for 5-day-old mosquitoes, 56 times lower than the ED_{50} for feeding. For 0-day-old mosquitoes, an almost identical model was calculated for the response of the sugar cell to sucrose presented in 10 mmol l^{-1} KCl and for sucrose presented in water. The sugar cell seems not to be affected by 10 mmol l^{-1} KCl, contrary to its effect on the water cell. Whereas *A. gambiae* is apparently sensitive to sucrose concentrations between 0.1 and 10 mmol l^{-1}, it does not necessarily imbibe such solutions of a suboptimal energy level. Mosquitoes probably use other taste sensilla during their probing-walking responses (Pappas and Larsen, 1978; Sanford and Tomberlin, 2011) to reach a source of sugar at 50 mmol l^{-1} or higher that activates the neurone near its maximum frequency.

In contrast to sucrose, solutions of glucose or fructose alone (the hexose sugars of sucrose) show, respectively, low and intermediate stimulatory effects on feeding. However, female *A. gambiae* engorge on glucose and fructose when combined at 146 mmol l^{-1} each, just as on 146 mmol l^{-1} sucrose, and the feeding response to this mixture is higher than on a 292 mmol l^{-1} solution of either glucose or fructose alone. A direct correlation exists between this feeding response and the sensory input in that an equimolar 146 mmol l^{-1} solution of glucose plus fructose activates the sucrose neurone to the same extent as either 146 mmol l^{-1} or 292 mmol l^{-1} sucrose, whereas no such activation is observed when the sensory neurone is stimulated with either hexose alone. This suggests a synergetic effect between glucose and fructose on the feeding response of female *A. gambiae* that is mediated by the sucrose taste neurone in the labellar trichoid T1 sensillum, a phenomenon already described for the blowfly *Phormia regina* (Dethier et al., 1956; Omand and Dethier, 1969) and the mosquitoes *Culiseta inornata* (Schmidt and Friend, 1991) and *A. aegypti* (Ignell et al., 2010). The fact that the sucrose labellar neurone of *A. gambiae* is activated by sucrose or by a mixture of glucose plus fructose but not by glucose or fructose alone suggests that it expresses distinct domains for glucose and fructose that both require activation to obtain a response from the neurone. It was proposed that the dendritic membrane of contact chemoreceptor cells in *C. inornata* is composed of pyranose sites specific for α-glucopyranose and fructopyranose, and fructofuranose sites (Schmidt and Friend, 1991). It is known that several Grs are co-expressed in insect contact chemoreceptor cells (Isono and Morita, 2010) and sugars are detected through multimeric receptors composed of two or more Grs in *Drosophila* (Dahanukar et al., 2007). Eight genes related to the *Gr5a* of *Drosophila* have already been identified as candidate sugar receptors in the *A. gambiae* genome (Hill et al., 2002; Kent et al., 2008). The low phagostimulatory effect of glucose and fructose on *A. gambiae* is not in agreement with what is known for other mosquito genera where fructose, glucose and sucrose all show strong phagostimulatory effects on their own on *C. inornata*, *A. aegypti* and *Aedes taeniorhynchus* (Wiedemann) (Salama, 1967; Nayar and Sauerman, 1971).

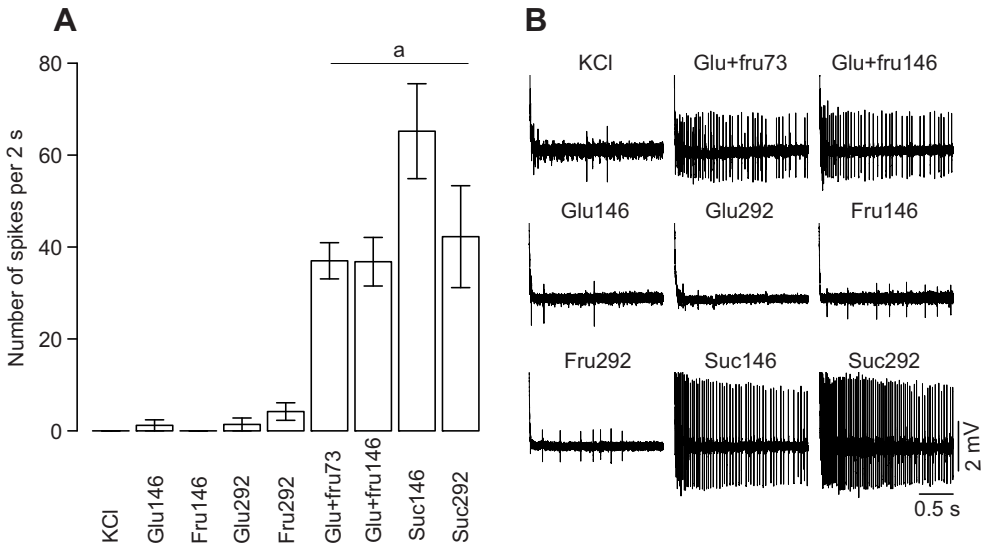

Fig. 14. (A) Mean ± s.e.m. number of spikes recorded from a T1 sensillum per 2 s to 10 mmol l^{-1} KCl and to 146 mmol l^{-1} glucose (glu146), 146 mmol l^{-1} fructose (fru146), 292 mmol l^{-1} glucose (glu292), 292 mmol l^{-1} fructose (fru292), 73 mmol l^{-1} glucose plus 73 mmol l^{-1} fructose (glu+fru73), 146 mmol l^{-1} glucose plus 146 mmol l^{-1} fructose (glu+fru146), 146 mmol l^{-1} sucrose (suc146) and 292 mmol l^{-1} sucrose (suc292) in 10 mmol l^{-1} KCl. Recordings were made from four to five female mosquitoes. The number of spikes per 2 s recorded for the mixtures of glucose plus fructose and to sucrose at the two concentrations tested is marginally not significant (a, ANOVA, P=0.06). (B) Corresponding electrophysiological recordings in response to stimulation with KCl, glucose, fructose, glucose plus fructose, and to sucrose.

Although mosquitoes can drink water to maintain their water balance (Benoit and Denlinger, 2010), the amount of pure water drunk in our feeding assay by mosquitoes maintained at a high relative humidity was low. In our assay, negative values for meal size are probably related to body water loss when the mosquitoes did not feed at all (Benoit et al., 2010). Our results suggest that the sugar receptor cells are responsible for food acceptance rather than the water neurones when mosquitoes are exposed to sucrose diluted in water. In the blowfly *Protophormia terraenovae* (Robineau-Desvoidy) it was shown that labellar water neurones function as osmometers since saline solutions, hypertonic with respect to the intracellular medium, cause cell shrinkage contrary to hypotonic solutions, such as that of glycerol, which cause cell swelling (Solari et al., 2010). Cell swelling coupled to a calcium influx leads to a transient receptor potential and action potential generation (Liscia et al., 1997). The possibility that the water receptor cell functions as an osmometer in *A. gambiae* is indicated by the strong inhibitory effect on the labellar hair neurone found here for $CuSO_4$ at 1 mmol l^{-1} (a supposed aquaglyceroporin AQP3 inhibitor) (Zelenina et al., 2004). A similar effect has been recorded for $CuSO_4$ on the water cell on the labellar lobes of *P. terraenovae* (Solari et al., 2010). Despite the strong inhibitory effect found here for $LaCl_3$ on the labellar water neurone of *A. gambiae*, a broad Ca^{2+}-permeable channel inhibitor, no significant activation of the neurone was observed when the extracellular Ca^{2+} concentration was increased by stimulating the sensillum with $CaCl_2$, as observed in the tarsal water neurones of *Drosophila* (Meunier et al., 2009). Whereas 1 mmol l^{-1} KCl is used as the reference to characterize the water cell in labellar and tarsal taste sensilla of *D. melanogaster* (Hiroi et al., 2004; Meunier et al., 2009), the labellar neurone of *Anopheles* appears to be more sensitive to KCl as this concentration reduced the number of spikes generated by the water cell of the T1 sensillar

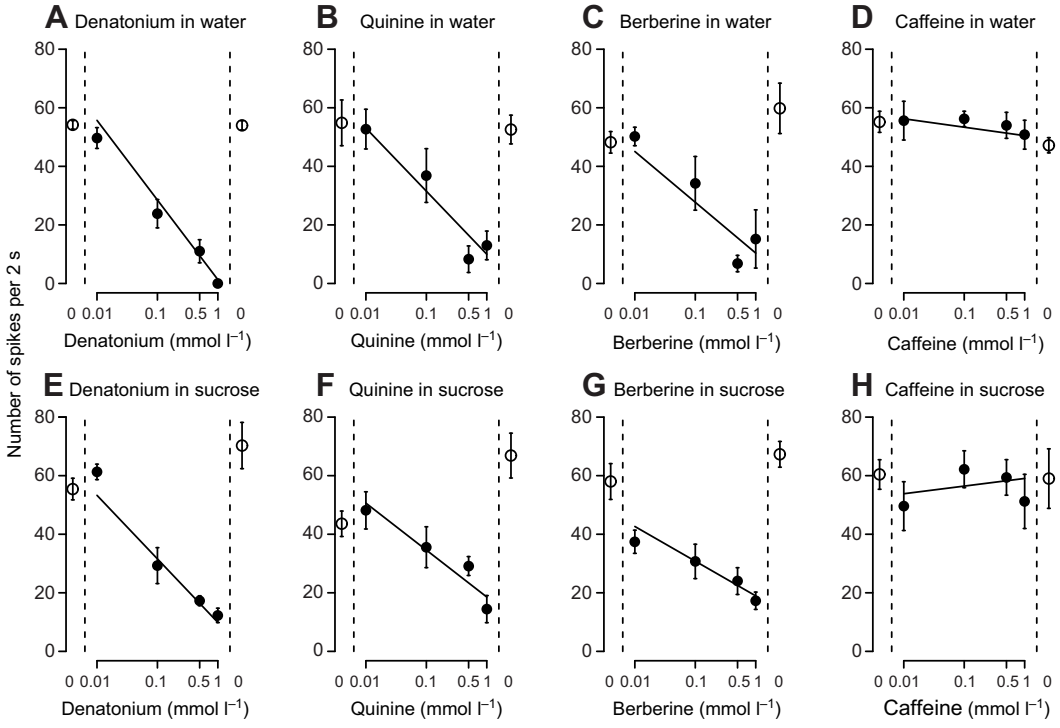

Fig. 15. Linear model ($F_{9,186}$=37.34, P<0.001, R^2= 0.63) and mean ± s.e.m. number of spikes generated per 2 s by the water cell (upper row of graphs) and by the sucrose cell in T1 sensilla (lower row of graphs) as a function of the $\log_{10}$ concentration of denatonium benzoate (six and seven mosquitoes tested), quinine (seven mosquitoes tested in both conditions), berberine (five and seven mosquitoes tested) and caffeine (five mosquitoes tested in both conditions) added to water (A–D) and to 146 mmol l^{-1} sucrose in 10 mmol l^{-1} KCl (E–H).

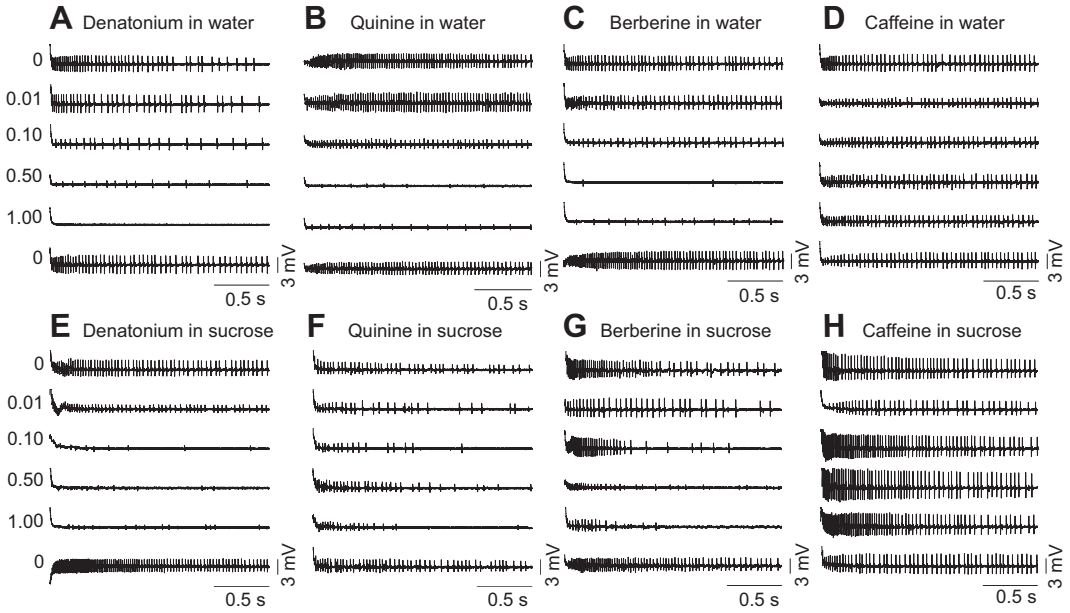

Fig. 16. Representative 2 s electrophysiological responses of the sucrose and water cells in T1 labellar sensilla of *A. gambiae* to water (A–D) and 146 mmol l^{-1} sucrose in 10 mmol l^{-1} KCl (E–H) each with increasing concentrations (0.01–1 mmol l^{-1}) of denatonium benzoate, quinine, berberine and caffeine added. Each recording series starts and ends with water (A–D) or 146 mmol l^{-1} sucrose in 10 mmol l^{-1} KCl (E–H). Except for caffeine, spike amplitude decreases with increasing concentration of all products tested.

receptor cells by 40%. Water, salt and sugar neurones have been identified in labellar trichoid T1 of *C. inornata* (Owen et al., 1974; Pappas and Larsen, 1976). The functionality of the lateral labellar T1 sensillum neurones in *A. gambiae* on which this study focused seems to be similar as separate sugar and water neurones have been identified in the present study. However, the functionality of the other labellar T1 sensilla of *A. gambiae* still need to be characterized.

Availability of sugars for *A. gambiae* in its habitat

Anopheles gambiae feeds preferentially on nectars from *Ricinus communis* (Euphorbiaceae) and *Tecoma stans* (Bignoniaceae) in its natural habitat (Manda et al., 2007b). In the case of *R. communis*, sucrose, glucose and fructose are present at a concentration of about 30% (w/v, 876 mmol l^{-1} for sucrose and 1665 mmol l^{-1} for the two hexoses) each in extrafloral nectar (Baker et al., 1978), and fructose and glucose are present at a concentration of around 50% (2775 mmol l^{-1}) each in floral nectar of *T. stans* whereas its sucrose content is only 2% (Freeman et al., 1985). The ratio between sucrose and hexose sugars can vary in floral nectar but it is rare to find nectar in which only one sugar occurs (Baker and Baker, 1982). Of the five Kenyan endemic plant species on which *A. gambiae* were observed feeding most frequently, fructose or glucose were never detected alone in floral or extra-floral nectaries (Manda et al., 2007a). In the same study these authors have shown that longevity in days of female *A. gambiae* that had access to 6% (w/v) glucose increased by more than fivefold over females that had access only to water. If *A. gambiae* is to exploit glucose or fructose alone as a resource, then individuals need to be starved for more than the 3 h period used in this study to compensate for the lower phagostimulatory effect of these monosaccharides. This is quite a plausible scenario since a steady diet of 10% sucrose during the days prior to feeding assays described here allowed *Anopheles* to increase its carbohydrate and lipid reserves (Briegel, 1990). Some plant nectars are more suitable at increasing *A. gambiae* longevity and fecundity over others (Manda et al., 2007a; Manda et al., 2007b). Such differences among plant species in meal quality could be due to optimal sugar levels combined with other essential nutrients or to the absence of toxic compounds, whereas plant preferences could be related to absence of deterrents.

Bitter products as feeding deterrents for *A. gambiae*

Except for caffeine, all bitter products tested significantly reduced sugar feeding in *A. gambiae*. The glass capillary feeding experiment including quinine allows us to conclude that proboscis and/or cibarial gustatory receptor cells are sufficient for the perception of bitter products. Ignell et al. (Ignell et al., 2010) found that the concentration of quinine that starts to inhibit feeding in *A. aegypti* is affected by the sucrose level presented, with only 0.001 mmol l^{-1} quinine required at 10 μmol l^{-1} sucrose but with 0.1 mmol l^{-1} required at 1 mol l^{-1} sucrose. The latter concentration of quinine is proportional to what starts to be effective at inhibiting feeding by *A. gambiae* in our no-choice assay, i.e. 0.01 mmol l^{-1} quinine in 146 mmol l^{-1} sucrose. However, whereas Ignell et al. (Ignell et al., 2010) found that quinine only affects feeding by female *A. aegypti*, quinine and quinidine were found to affect the feeding response of male and female *A. gambiae* in a comparable manner in our assays (data not shown). In most cases alkaloids inhibit feeding in insects (Dethier and Bowdan, 1989; Ramaswamy et al., 1992; Zhang and Mitchell, 1997; Meunier et al., 2003). Bitter products are not all equally toxic, and, to date, it is not clear if there is a direct correlation between bitterness and toxicity of these products for insects (Glendinning, 2002; Weiss et al., 2011).

Bitter products inhibit sucrose-feeding in *Anopheles* through sensory inhibition

The concentration-dependent inhibitory effects of denatonium benzoate, quinine and berberine on the electrophysiological responses of labellar sucrose and water receptor cells to water or sucrose confirms the critical role of sensory inhibition in the perception of deterrent compounds in *A. gambiae* as in other insects (Schoonhoven, 1982). These compounds also inhibit feeding, whereas caffeine, which fails to inhibit feeding even at 1 mmol l^{-1} by *A. gambiae* on sucrose, shows no inhibitory effect on the electrophysiological responses of the neurones. Inhibition of the receptor cell responses is stronger when denatonium benzoate, quinine and berberine are presented in pure water than in 146 mmol l^{-1} sucrose, indicative of a competitive phenomenon between sucrose and these bitter products. This suggests that the bitter compounds induce inhibition of the sucrose cell by acting on specific Gr(s) or on a specific ion channel type. It was suggested

that the water receptor cells in *Drosophila* are inhibited by bitter products through an osmolarity-based mechanism (Meunier et al., 2003; Weiss et al., 2011). In our study we show that both the labellar sucrose and water neurones would appear to be involved in the detection of all products showing feeding deterrent activity in *A. gambiae*. Nevertheless, the numbers of bitter compounds we have tested is not exhaustive. To date, we have not succeeded in characterizing receptor cells in sensilla on the proboscis or on the tarsi of *A. gambiae* that are selectively activated by bitter products, as is known in *Drosophila* (Meunier et al., 2003; Hiroi et al., 2004; Weiss et al., 2011). It has recently been shown that labellar neurones responding to bitter products in *Drosophila* can be segregated into four functional classes, each possessing a specific response pattern to 16 bitter products and expressing a specific combination of 33 Grs (Weiss et al., 2011).

In summary, this study highlights how bitter-tasting products have a deterrent effect on sugar feeding by *A. gambiae*. The diversity of bitter compounds in nature has led to a wide diversity of Grs involved in the detection of unpalatable substances in *Drosophila* (Weiss et al., 2011). Despite this, we have identified two neurones on the labellar lobes of *A. gambiae* that are activated by palatable compounds and inhibited by unpalatable ones. These neurones could be the target of future tests to uncover novel deterrents for *A. gambiae* and to identify phagostimulants to better understand the ecology of feeding in this species.

LIST OF ABBREVIATIONS

ED_{50}	effective dose that induces 50% of the maximum response
MMS	median meal size
MNS	mean number of spikes per 2 s generated by only one receptor cell
MTNoS	mean of the total number of spikes per 2 s counted without discriminating between the receptor cells of the sensillum

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REFERENCES

Arsic, D. and Guerin, P. M. (2008). Nutrient content of diet affects the signaling activity of the insulin/target of rapamycin/p70 S6 kinase pathway in the African malaria mosquito *Anopheles gambiae*. *J. Insect Physiol.* **54**, 1226-1235.

Baker, H. G. and Baker, I. (1982). Floral nectar sugar constituents in relation to pollinator type. In *Handbook of Experimental Pollination Biology* (ed. C. E. Jones and R. J. Little), pp. 117-141. New York, NY: Van Nostrand Reinhold.

Baker, D. A., Hall, J. L. and Thorpe, J. R. (1978). A study of extrafloral nectaries of *Ricinus communis*. *New Phytol.* **81**, 129-137.

Benoit, J. B. and Denlinger, D. L. (2010). Meeting the challenges of on-host and off-host water balance in blood-feeding arthropods. *J. Insect Physiol.* **56**, 1366-1376.

Benoit, J. B., Lopez-Martinez, G., Phillips, Z. P., Patrick, K. R. and Denlinger, D. L. (2010). Heat shock proteins contribute to mosquito dehydration tolerance. *J. Insect Physiol.* **56**, 151-156.

Briegel, H. (1990). Fecundity, metabolism, and body size in *Anopheles* (Diptera: Culicidae), vectors of malaria. *J. Med. Entomol.* **27**, 839-850.

Dahanukar, A., Foster, K., van der Goes van Naters, W. M. and Carlson, J. R. (2001). A Gr receptor is required for response to the sugar trehalose in taste neurons of *Drosophila*. *Nat. Neurosci.* **4**, 1182-1186.

Dahanukar, A., Lei, Y. T., Kwon, J. Y. and Carlson, J. R. (2007). Two Gr genes underlie sugar reception in *Drosophila*. *Neuron* **56**, 503-516.

Dethier, V. G. and Bowdan, E. (1989). The effect of alkaloids on sugar receptors and the feeding behaviour of the blowfly. *Physiol. Entomol.* **14**, 127-136.

Dethier, V. G. and Bowdan, E. (1992). Effects of alkaloids on feeding by *Phormia regina* confirm the critical role of sensory inhibition. *Physiol. Entomol.* **17**, 325-330.

Dethier, V. G., Evans, D. R. and Rhoades, M. V. (1956). Some factors controlling the ingestion of carbohydrates by the blowfly. *Biol. Bull.* **111**, 204-222.

Foster, W. A. (1995). Mosquito sugar feeding and reproductive energetics. *Annu. Rev. Entomol.* **40**, 443-474.

Foster, W. A. and Takken, W. (2004). Nectar-related vs. human-related volatiles: behavioural response and choice by female and male *Anopheles gambiae* (Diptera: Culicidae) between emergence and first feeding. *Bull. Entomol. Res.* **94**, 145-157.

Freeman, C. E., Worthington, R. D. and Corral, R. D. (1985). Some floral nectar-sugar compositions from Durango and Sinaloa, Mexico. *Biotropica* **17**, 309-313.

Fujishiro, N., Kijima, H. and Morita, H. (1984). Impulse frequency and action potential amplitude in labellar chemosensory neurones of *Drosophila melanogaster*. *J. Insect Physiol.* **30**, 317-325.

Gary, R. E., Jr and Foster, W. A. (2001). Effects of available sugar on the reproductive fitness and vectorial capacity of the malaria vector *Anopheles gambiae* (Diptera: Culicidae). *J. Med. Entomol.* **38**, 22-28.

Gary, R. E., Jr., Cannon, J. W., III and Foster, W. A. (2009). Effect of sugar on male *Anopheles gambiae* mating performance, as modified by temperature, space, and body size. *Parasit. Vectors* **2**, 19.

Glendinning, J. I. (2002). How do herbivorous insects cope with noxious secondary plant compounds in their diet? *Entomol. Exp. Appl.* **104**, 15-25.

Heitler, W. J. (1999). DataView version 8.0.0. St Andrews, UK: University of St Andrews. Available at: www.st-andrews.ac.uk/~wjh/dataview/.

Hill, S. R. and Smith, B. J. J. (1999). Consistent pattern in the placement of taste sensilla on the labellar lobes of *Aedes aegypti*. *Int. J. Insect Morphol. Embryol.* **28**, 281-290.

Hill, C. A., Fox, A. N., Pitts, R. J., Kent, L. B., Tan, P. L., Chrystal, M. A., Cravchik, A., Collins, F. H., Robertson, H. M. and Zwiebel, L. J. (2002). G protein-coupled receptors in *Anopheles gambiae*. *Science* **298**, 176-178.

Hiroi, M., Meunier, N., Marion-Poll, F. and Tanimura, T. (2004). Two antagonistic gustatory receptor neurons responding to sweet-salty and bitter taste in *Drosophila*. *J. Neurobiol.* **61**, 333-342.

Hodgson, E. S., Lettvin, J. Y. and Roeder, K. D. (1955). Physiology of a primary chemoreceptor unit. *Science* **122**, 417-418.

Ignell, R. and Hansson, B. S. (2005). Projection patterns of gustatory neurons in the suboesophageal ganglion and tritocerebrum of mosquitoes. *J. Comp. Neurol.* **492**, 214-233.

Ignell, R., Okawa, S., Englund, J. E. and Hill, S. R. (2010). Assessment of diet choice by the yellow fever mosquito *Aedes aegypti*. *Physiol. Entomol.* **35**, 274-286.

Isono, K. and Morita, H. (2010). Molecular and cellular designs of insect taste receptor system. *Front. Cell. Neurosci.* **4**, 20.

Jiao, Y., Moon, S. J. and Montell, C. (2007). A *Drosophila* gustatory receptor required for the responses to sucrose, glucose, and maltose identified by mRNA tagging. *Proc. Natl. Acad. Sci. USA.* **104**, 14110-14115.

Kaissling, K.-E. (1995). Single unit and electroantennogram recordings in insect olfactory organs. In *Experimental Cell Biology of Taste and Olfaction* (ed. A. I. Spielman and J. G. Brand), pp. 361-377. Boca Raton, FL: CRC Press.

Kent, L. B., Walden, K. K. O. and Robertson, H. M. (2008). The Gr family of candidate gustatory and olfactory receptors in the yellow-fever mosquito *Aedes aegypti*. *Chem. Senses* **33**, 79-93.

Liscia, A., Solari, P., Majone, R., Tomassini Barbarossa, I. and Crnjar, R. (1997). Taste reception mechanisms in the blowfly: evidence of amiloride-sensitive and insensitive receptor sites. *Physiol. Behav.* **62**, 875-879.

Magnarelli, L. A. (1979). Diurnal nectar-feeding of *Aedes cantator* and *A. sollicitans* (Diptera, Culicidae). *Environ. Entomol.* **8**, 949-955.

Manda, H., Gouagna, L. C., Foster, W. A., Jackson, R. R., Beier, J. C., Githure, J. I. and Hassanali, A. (2007a). Effect of discriminative plant-sugar feeding on the survival and fecundity of *Anopheles gambiae*. *Malar. J.* **6**, 113.

Manda, H., Gouagna, L. C., Nyandat, E., Kabiru, E. W., Jackson, R. R., Foster, W. A., Githure, J. I., Beier, J. C. and Hassanali, A. (2007b). Discriminative feeding behaviour of *Anopheles gambiae* s.s. on endemic plants in western Kenya. *Med. Vet. Entomol.* **21**, 103-111.

Marion-Poll, F. and van der Pers, J. (1996). Un-filtered recordings from insect taste sensilla. *Entomol. Exp. Appl.* **80**, 113-115.

Meunier, N., Marion-Poll, F., Rospars, J. P. and Tanimura, T. (2003). Peripheral coding of bitter taste in *Drosophila*. *J. Neurobiol.* **56**, 139-152.

Meunier, N., Marion-Poll, F. and Lucas, P. (2009). Water taste transduction pathway is calcium dependent in *Drosophila*. *Chem. Senses* **34**, 441-449.

Moon, S. J., Köttgen, M., Jiao, Y. C., Xu, H. and Montell, C. (2006). A taste receptor required for the caffeine response *in vivo*. *Curr. Biol.* **16**, 1812-1817.

Nayar, J. K. and Sauerman, D. M., Jr (1971). Physiological effects of carbohydrates on survival, metabolism, and flight potential of female *Aedes taeniorphynchus*. *J. Insect Physiol.* **17**, 2221-2233.

Odalo, J. O., Omolo, M. O., Malebo, H., Angira, J., Njeru, P. M., Ndiege, I. O. and Hassanali, A. (2005). Repellency of essential oils of some plants from the Kenyan coast against *Anopheles gambiae*. *Acta Trop.* **95**, 210-218.

Omand, E. and Dethier, V. G. (1969). An electrophysiological analysis of the action of carbohydrates on the sugar receptor of the blowfly. *Proc. Natl. Acad. Sci. USA.* **62**, 136-143.

Owen, W. B. (1971). Taste receptors of the mosquito *Anopheles atroparvus* Van Thiel. *J. Med. Entomol.* **8**, 491-494.

Owen, W. B., Larsen, J. R. and Pappas, L. G. (1974). Functional units in the labellar chemosensory hairs of the mosquito *Culiseta inornata* (Williston). *J. Exp. Zool.* **188**, 235-247.

Pappas, L. G. and Larsen, J. R. (1976). Gustatory hairs on the mosquito, *Culiseta inornata*. *J. Exp. Zool.* **196**, 351-360.
Pappas, L. G. and Larsen, J. R. (1978). Gustatory mechanisms and sugar-feeding in the mosquito, *Culiseta inornata*. *Physiol. Entomol.* **3**, 115-119.
Petanidou, T. and Lamborn, E. (2005). A land for flowers and bees: studying pollination ecology in Mediterranean communities. *Plant Biosyst.* **139**, 279-294.
R Development Core Team (2010). *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.
Ramaswamy, S. B., Cohen, N. E. and Hanson, F. E. (1992). Deterrence of feeding and oviposition responses of adult *Heliothis virescens* by some compounds bitter-tasting to humans. *Entomol. Exp. Appl.* **65**, 81-93.
Rasband, W. S. (2011). ImageJ version 1.44p. Bethesda, MD: US National Institutes of Health. Available at: http://imagej.nih.gov/ij/.
Salama, H. S. (1967). Nutritive values and taste sensitivity to carbohydrates for mosquitoes. *Mosq. News* **27**, 32-35.
Sanford, M. R. and Tomberlin, J. K. (2011). Conditioning individual mosquitoes to an odor: sex, source, and time. *PLoS ONE* **6**, e24218.
Schmidt, J. M. and Friend, W. G. (1991). Ingestion and diet destination in the mosquito *Culiseta inornata*: effects of carbohydrate configuration. *J. Insect Physiol.* **37**, 817-828.
Schoonhoven, L. M. (1982). Biological aspects of antifeedants. *Entomol. Exp. Appl.* **31**, 57-69.
Sellier, M. J., Reeb, P. and Marion-Poll, F. (2011). Consumption of bitter alkaloids in *Drosophila melanogaster* in multiple-choice test conditions. *Chem. Senses* **36**, 323-334.
Slone, J., Daniels, J. and Amrein, H. (2007). Sugar receptors in *Drosophila*. *Curr. Biol.* **17**, 1809-1816.
Solari, P., Masala, C., Falchi, A. M., Sollai, G. and Liscia, A. (2010). The sense of water in the blowfly *Protophormia terraenovae*. *J. Insect Physiol.* **56**, 1825-1833.
Timmermann, S. E. and Briegel, H. (1993). Water depth and larval density affect development and accumulation of reserves in laboratory populations of mosquitoes. *Bull. Soc. Vector Ecol.* **18**, 174-187.
Van Handel, E. (1984). Metabolism of nutrients in the adult mosquito. *Mosq. News* **44**, 573-579.
Weiss, L. A., Dahanukar, A., Kwon, J. Y., Banerjee, D. and Carlson, J. R. (2011). The molecular and cellular basis of bitter taste in *Drosophila*. *Neuron* **69**, 258-272.
Wykes, G. R. (1952). An investigation of the sugars present in the nectar of flowers of various species. *New Phytol.* **51**, 210-215.
Yuval, B., Hollidayhanson, M. L. and Washino, R. K. (1994). Energy budget of swarming male mosquitoes. *Ecol. Entomol.* **19**, 74-78.
Zelenina, M., Tritto, S., Bondar, A. A., Zelenin, S. and Aperia, A. (2004). Copper inhibits the water and glycerol permeability of aquaporin-3. *J. Biol. Chem.* **279**, 51939-51943.
Zhang, T. Z. and Mitchell, B. K. (1997). Components of tomato leaf homogenate suppress responses from galeal chemoreceptors of the adult Colorado potato beetle. *Physiol. Entomol.* **22**, 291-296.

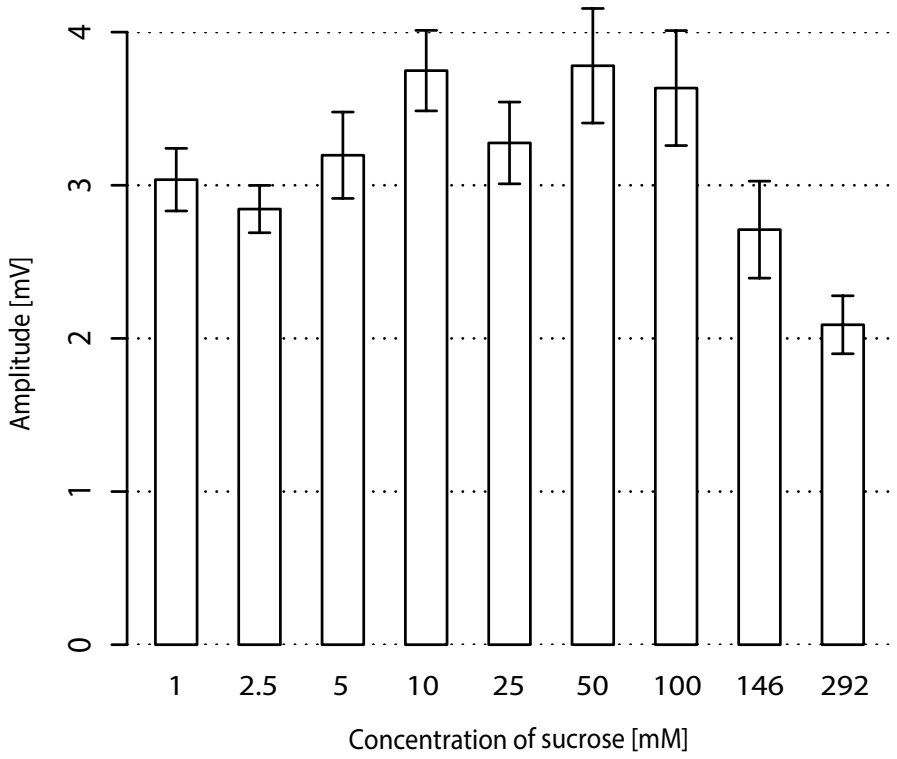

Fig. S1. Mean ± s.e.m. spike amplitudes generated by the sucrose-sensitive cell to increasing concentrations of sucrose in 10 mmol l^{-1} KCl (data set as in Fig. 10).

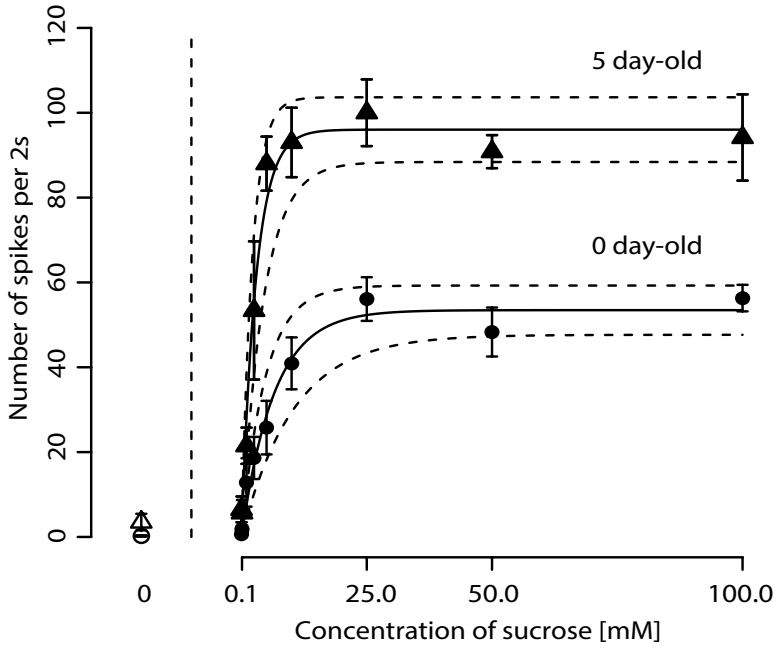

Fig. S2. Asymptotic models of the number of spikes (mean ± s.e.m.) per 2 s emitted by the sucrose-sensitive receptor cell in trichoid T1 sensilla on the labellum of 0-day-old (circles) and 5-day-old (triangles) female *A. gambiae* to stimulation with 0, 0.01, 0.1, 1, 2.5, 5, 10, 25, 50 and 100 mmol l^{-1} sucrose presented in increasing concentrations in water. The dotted lines indicate the 95% confidence intervals of the models. The response to water alone (open symbols on left) is not taken into account in the models (five to 13 mosquitoes tested per concentration, data sets as in Fig. 12).

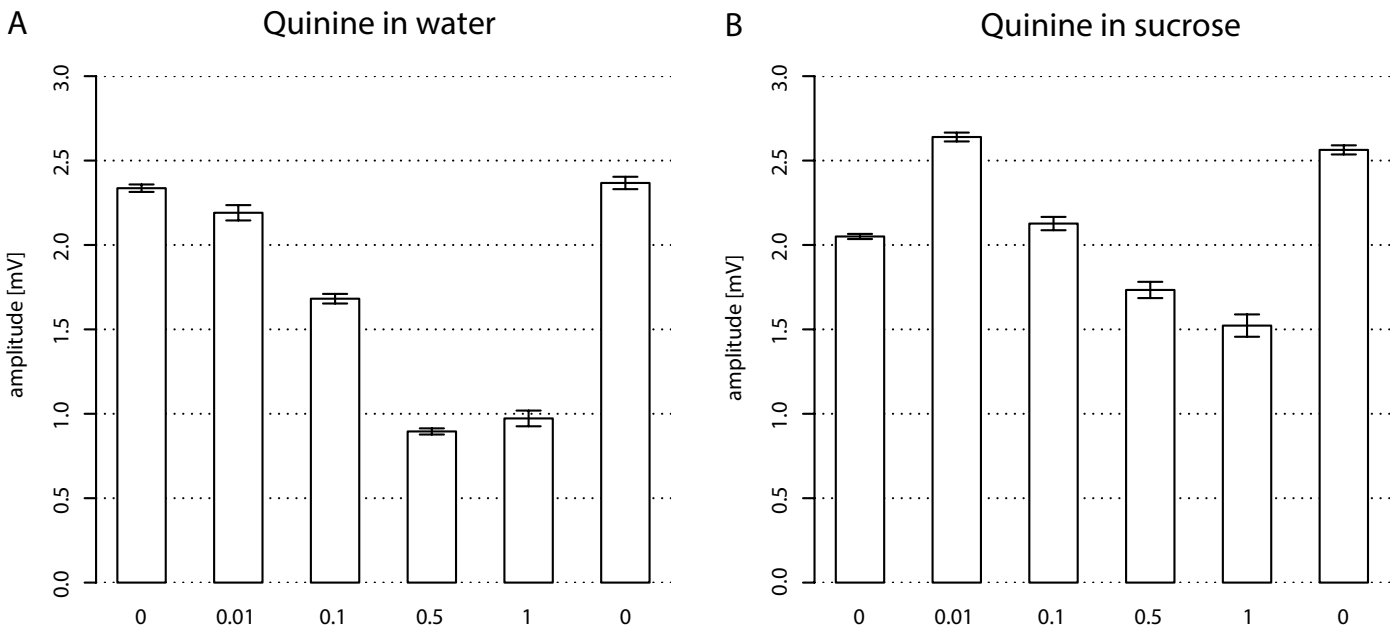

Fig. S3. Mean ± s.e.m. spike amplitudes of the water receptor cell (A) and the sucrose receptor cell (B) as a function of increasing concentrations of quinine (data sets as in Fig. 15B,F).

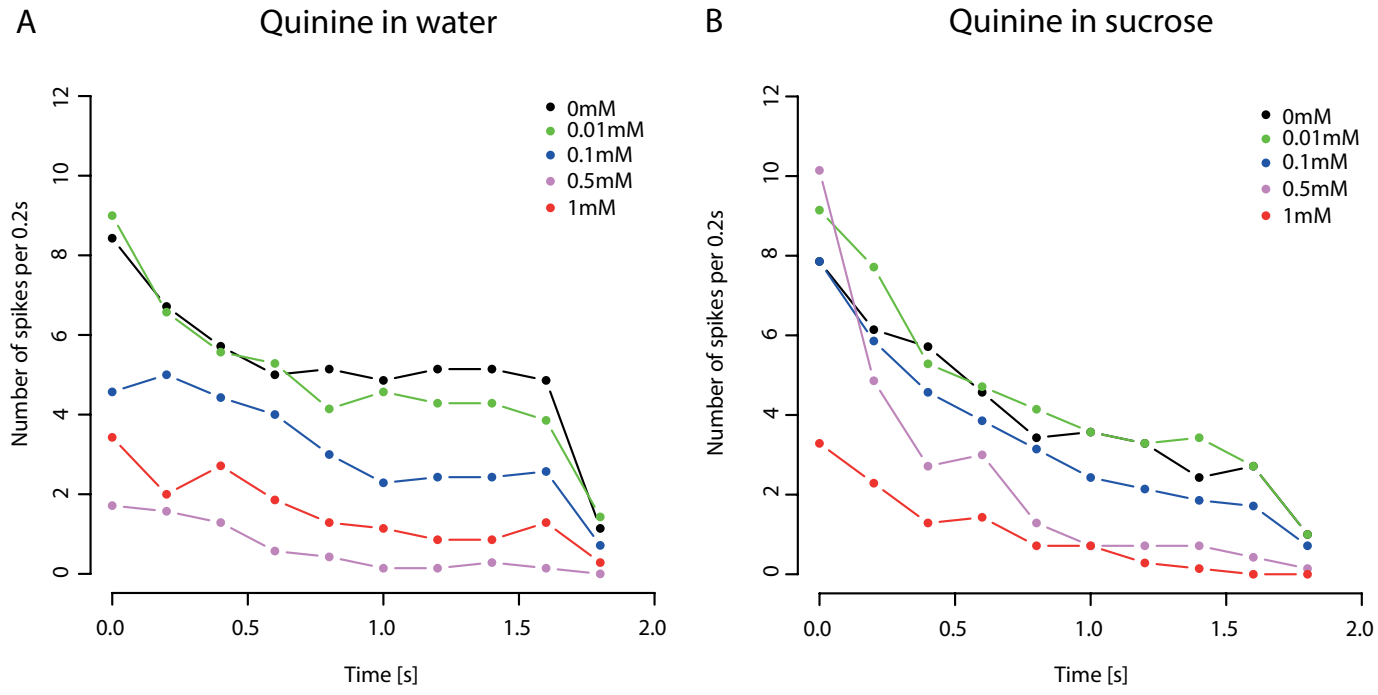

Fig. S4. Mean number of action potentials per 0.2 s in 2 s recordings from the water cell (A) and the sucrose cell (B) for 0, 0.01, 0.1, 0.5 and 1 mmol l^{-1} quinine diluted in water (A) and in 5% sucrose plus 10 mmol l^{-1} KCl (B) (data sets as in Fig. 15B,F).