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Olfactory Coding in Antennal Neurons of the Malaria Mosquito, *Anopheles gambiae*

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Abstract

Olfactory receptor neurons (ORNs) in the antenna of insects serve to encode odors in action potential activity conducted to the olfactory lobe of the deuterocerebrum. We performed an analysis of the electrophysiological responses of olfactory neurons in the antennae of the female malaria mosquito *Anopheles gambiae* s.s. and investigated the effect of blood feeding on responsiveness. Forty-four chemicals that are known to be present in human volatile emanations were used as odor stimuli. We identified 6 functional types of trichoid sensilla and 5 functional types of grooved-peg sensilla (GP) based on a hierarchical cluster analysis. Generalist ORNs, tuned to a broad range of odors, moderate specialist ORNs and 2 ORNs tuned to only one odor were identified in different sensilla types. Neurons in GP were tuned to more polar compounds including the important behavioral attractant ammonia and its synergist L-lactic acid, responses to which were found only in GP. Combinatorial coding is the most plausible principle operating in the olfactory system of this mosquito species. We document for the first time both up- and downregulation of ORN responsiveness after blood feeding. Modulation of host-seeking and oviposition behavior is associated with both qualitative and quantitative changes in the peripheral sensory system.

Key words: electrophysiology, human odor, olfaction, ORN, receptor neuron, single-sensillum recording

Introduction

Insects rely to a large extent on olfactory information to locate food, mating partners, and breeding sites (Hildebrand and Shepherd 1997). Olfactory receptor neurons (ORNs) in insects are contained in sensilla and cuticular extensions of various shapes predominantly present on their antennae and mouthparts. ORNs have been shown to encode odor quality, quantity, and temporal changes in odor concentration (Heinbockel and Kaissling 1996; De Bruyne et al. 1999, 2001; Mustaparta 2002).

Anopheles gambiae is the main vector of malaria in Africa, causing more than one million victims each year (Snow et al. 2005). Being nocturnal, a female *A. gambiae* mosquito is guided to its human hosts predominantly by olfactory cues (Takken 1991). The recent sequencing of the complete genome of *A. gambiae* (Holt et al. 2002), containing at least 79 putative olfactory receptor (OR) genes (Hill et al. 2002), has led to its adoption as a new model organism for the study of the olfactory system.

The antennae of female *A. gambiae* mosquitoes carry 4 types of sensilla: trichoid sensilla (sensilla trichodea), grooved-peg sensilla (GP), sensilla coeloconica, and sensilla ampullacea (Figure 1). The latter 2 types are present in low numbers on the antennae, and they are possibly innervated by thermoreceptor neurons (Davis and Sokolove 1975; McIver 1982). Each antenna of female *A. gambiae* mosquitoes bears about 630 trichoid sensilla and 84 GP, both types containing an estimated total of 1500–1600 ORNs per antenna (Ismail 1964; Van Den Broek and Den Otter 1999; Meijerink et al. 2001). Trichoid sensilla are single-walled wall pore sensilla and GP are double walled with 10–12 hollow finger-like structures fused to each other (Steinbrecht 1997; Pitts and Zwiebel 2006).

ORNs in trichoid and GP project to different glomerular areas of the antennal lobe, the primary integration center in the deuterocerebrum (Anton et al. 2003; Anton and Rospars 2004). Carbon dioxide–sensitive neurons contained in sensilla on the maxillary palp also project to the antennal lobe, but to a different area (Anton et al. 2003).

Behavioral studies have demonstrated that various human skin emanations or skin secretions attract female *A. gambiae*

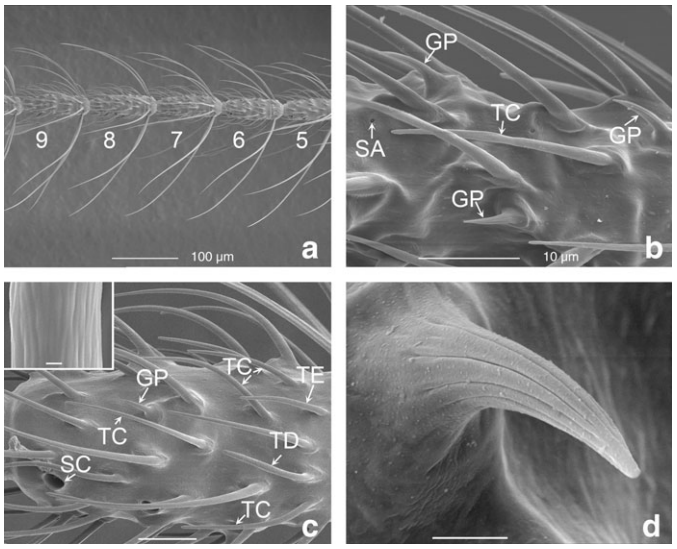

Figure 1 Scanning electron micrographs of antennal sensilla of a female mosquito of *Anopheles gambiae*. **(a)** the 5th to 9th antennomeres of a female *A. gambiae*. Bar = 100 µm. **(b)** detail showing GP, TC, and sensilla ampullacea (SA). Bar = 10 µm. **(c)** TC, TD, and TE; sensilla coeloconica (SC). Bar = 10 µm; Inset, wall structure of TC showing slitlike structures on the surface. Bar = 0.1 µm. **(d)** GP. Bar = 1 µm.

mosquitoes (Braks and Takken 1999; Healy and Copland 2000; Pates et al. 2001). Attraction to several single chemical components of human origin has been documented for *A. gambiae*. Ammonia on its own was attractive in the olfactometer (Braks and Takken 1999; Smallegange et al. 2005). Recently, a tripartite synergism was demonstrated among ammonia, L-lactic acid, and a mixture of 12 carboxylic acids that occur in human emanations (Smallegange et al. 2005). Female mosquitoes need to take blood meals for egg development. Takken et al. (2001) found that host-seeking behavior of *A. gambiae* mosquitoes was suppressed after a blood meal and restored 24 h later. Electroantennogram recordings provided evidence that ORN sensitivity changed after a blood meal.

In this paper, we identify 6 functional groups of trichoid and 5 functional groups of GP based on responses to a panel of 44 compounds, including all compounds that have thus far been documented to elicit behavioral responses in *A. gambiae*. We also investigated the effect of blood feeding on specificity and sensitivity of trichoid sensilla to understand sensory modulation associated with switching between host-seeking and oviposition behavior.

Materials and methods

Insects

Anopheles gambiae sensu stricto, originated from Suakoko, Liberia (by courtesy of Professor M Coluzzi, Rome), were reared in the laboratory according to a standard protocol (Qiu et al. 2004). Electrophysiological recordings were taken from female mosquitoes that were 5–8 days old and either had only access to a 6% glucose solution or were allowed to take a full blood meal, as judged by the appearance of diuretic droplets, from a human arm. Adults that did not take a blood meal were removed from the cage. Blood-fed mosquitoes were kept in the climate room under the same conditions as the mosquito colony (with access to 6% glucose) for 2–24 h post-blood meal until they were subjected to recording.

After the legs had been removed, a female mosquito was attached to a transparent perspex block (1.1 × 1.1 × 1.5 cm) by a piece of transparent Scotch double-sided sticky tape (3M, Leiden, The Netherlands). The wings, mouthparts, and each junction between antennal segments were pressed gently against the tape to immobilize the mosquito. The antennae of the mosquito were viewed with an Olympus CK2 inverted microscope at 600× magnification. The lengths of trichoid sensilla were measured by a calibrated graded scale placed in the ocular of the microscope. Single-sensillum recordings were made from short- and medium-length trichoid and GP on segments 6–13 of the antenna that consists of 13 segments. We focussed on these sensilla because the success rate of recording responses to the tested stimuli was highest for these sensilla. The short- and medium-length trichoid sensilla morphologically resemble the trichoid subtypes E and C according to the classification for antennal sensilla presented by Boo (1980) for *Anopheles stephensi* and were named thereafter. There are about 30 trichoid E and 42 trichoid C sensilla on these segments.

Cryoscanning electron microscopy

Adult female mosquitoes were sedated by cooling on ice, mounted on a specimen holder using carbon adhesive tabs (Electron Microscopy Science, Washington), and quickly frozen in liquid nitrogen. The frozen samples were placed in a dedicated cryopreparation chamber (Oxford Instruments, CT 1500 HF, Eynsham, UK), in which the samples were freeze dried for 3 min at −90 °C at 8×10^{-4} Pa to remove water vapor. Afterward the samples were sputter coated with a layer of 15 nm platinum at the same temperature. The sample was cryotransferred into the field emission scanning microscope (JEOL 6300F, Tokyo, Japan) on a sample stage at −190 °C. The analysis was performed at a working distance of 16 mm, with secondary electron detection at 5 kV. All images were recorded digitally (Orion, 6 E.L.I. sprl, Charleroi, Belgium) at a scan rate of 100 s (full frame) at a size of 2528 × 2030, 8 bits. The images were optimized and resized with Adobe Photoshop CS.

Single-sensillum recording methods

Action potentials were recorded with a tungsten microelectrode (0.1 mm shaft diameter, World Precision Instruments, Berlin, Germany). The microelectrode was electrolytically sharpened to a tip diameter smaller than 1 µm by repeated

dipping into a saturated KNO_2 solution at a constant voltage of 4 V. The recording electrode was positioned at the base of a sensillum (Den Otter et al. 1980). An electronic micromanipulator (Micromanipulator 5170, Eppendorf-Netheler-Hinz, Hamburg, Germany) was used to move the recording electrode to a position at which electrophysiological activity was recorded. The signals were digitized by a USB-IDAC analog–digital conversion interface (Syntech, Hilversum, The Netherlands) at a sample rate of 11 900/s and amplified 1024×. Autospike software (Syntech) was used for both recording and data analysis.

The response of a neuron to a stimulus puff is quantified as the average action potential firing frequency in the first 0.5 s after the onset of a 0.2-s stimuli puff minus the average firing frequency in the 6 intervals of 0.5 s preceding stimulus delivery. The criteria for excitation or inhibition responses were an increase or decrease, respectively, of action potential frequency greater than 11 action potentials per second, which was twice the standard deviation (SD) of the average spontaneous activity. Action potentials from different neurons were sorted visually based on 1) discrete classes observed in the amplitude histogram produced by Autospike, 2) wave form, and 3) the occurrence of interspike intervals shorter than the refractory period (3.5 ms) (Lewicki 1998; Meunier et al. 2003). To evaluate the accuracy of our classification method, the spike numbers manually counted for responses of neurons in trichoid sensilla type E and C to indole, 4-methyl phenol, and geranyl acetone were compared with those estimated by a recently proposed algorithm. The activities of each single neuron in recordings in which 2 neurons are active were calculated. Based on the ''silent period'' and number of doublets, pairs of spikes were separated by a time interval shorter than the refractory period (Meunier et al. 2003). The spike frequencies resulting from visual sorting were statistically similar to those estimated by the algorithm (Wilcoxon signed ranks test, $P > 0.05$).

Anopheles gambiae has proven to be a difficult insect species for electrophysiological studies due to the low signal-to-noise ratio recorded using the surface-contact method employed. The amplitude of the action potentials was 0.2–0.4 mV, a value about 10 times lower than action potentials of ORNs from flies (fruit fly, house fly [Kelling et al. 2002], tsetse flies [Van Der Goes Van Naters et al. 1996]) or moths (Shields and Hildebrand 2001). The overall success rate of extracellular recordings from the antennal neurons of this species is around 10%.

Odor stimulation

A charcoal-filtered and humidified airstream (40 ml/s) was passed constantly over the mosquito antenna. Test compounds were dissolved in tertyl-butyl methyl ether (TBME, also known as MTBE), except for ammonia that was dissolved in water. A piece of filter paper (1 × 1.5 cm), onto which 25 µl of a solution of the test odor was dosed, was inserted into a Pasteur pipette; TBME was allowed to evaporate for 15 min before use. Filter paper with water and TBME treated in the same way as those carrying the odor solutions were used as blank controls. The odor vapor emanating from the solution on filter paper in the Pasteur pipette was injected into the main airstream using a stimulus controller (C5-01/b, Syntech); the flow of the airstream carrying the stimulus was set at 6.7 ml/s, producing a stimulus puff of 1.3 ml, while keeping the total flow constant.

Chemicals were of the highest purity grade commercially available: most of them were 95% to >99% pure. Aqueous solutions of L(+)-lactic acid (90% aqueous solution, pharmaceutical grade; >95% L-isomer) and ammonia (25% aqueous solution, analytical grade) were used. Most chemicals were purchased from Sigma-Aldrich and Fluka, except the following: L-lactic acid (Purac, Gorinchem, The Netherlands) and 3-methyl butanoic acid (Acros, 's-Hertogenbosch, The Netherlands). Two compounds, 7-octenoic acid and 3-methyl-2-hexenoic acid (both >99% pure) were kindly supplied by Dr M Birkett (IACR-Rothamsted, Harpenden, Hertfordshire, UK). Stimuli were tested in a random order; lower concentrations of stimuli were tested first to prevent possible adaptation to higher dosages. A 10^{-2} dilution (v/v for liquid or w/v for solid) of 44 compounds was used to screen the response spectrum of a sensillum. The 2-oxocarboxylic acids tested were found in human blood and urine (Healy and Copland 2000); 7-octenoic acid and 3-methyl-2-hexenoic acid were found in human axillary odors (Zeng et al. 1996; Healy et al. 2002); esters were components of odors from mosquito oviposition sites (Du and Millar 1999); the other compounds were all found in human skin emanations (Bernier et al. 2000; Meijerink et al. 2000; AM Galimard, unpublished data). The compounds for which behavioral activity for *A. gambiae* has been documented are indicated in Table 1.

Statistical analysis

Hierarchical cluster analysis using SPSS software package (release 11.0.1, SPSS Inc., Chicago, IL) was applied to classify the olfactory neurons based on their responses to a subset of compounds. Classification of neurons was based on response frequencies (positive value for excitation, negative value for inhibition) to several compounds. The subset of compounds was selected based on the observation that they elicited responses from some neurons and failed to do so from others (De Bruyne et al. 2001; Shields and Hildebrand 2001). For trichoid sensilla compounds included in the cluster analysis were indole, geranyl acetone, 4-ethyl phenol, and hexanoic acid. The compounds used for classification of neurons in GP were L-lactic acid, 2-oxobutanoic acid, and hexanoic acid. The distances between data points were calculated according to Ward's method (Ward 1963) and individual neurons were grouped based on the distances. The optimal number of groups was related to the largest distance

Table 1 Response spectra of each ORN class innervating TE and TC before and after blood meal (pTE and pTC)

Compounds	TE1		TE2		TC1		TC2		TC3		TC4		pTE1		pTE2		pTE3	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Ammonia and amines																		
Ammonia[a,b]	●	●	●	●	●	●	○	○	○	○	●	○	●	●	●	●	●	○
1-Butylamine	○	○			○	○	○	○	○	○	○	○					○	○
1-Pentylamine	○	○			○	○	○	○	○	○	○	○					●	□
Oxocarboxylic acids																		
2-Oxobutanoic acid[c,h]	○	○	●	●	○	○	○	○	○	○	○	○	○	○	○	○	○	○
2-Oxopentanoic acid[c,h]	○	○	●	●	○	○	○	○	○	○	○	○	○	○	○	○	○	○
2-Oxohexanoic acid[c,h]	○	○	○	○	○	○	○	○	○	○	○	○	○	○			○	○
Carboxylic acids																		
L(+) Lactic acid[a,b,d,h]	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Acetic acid	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Propanoic acid[e,h]	□	○	●	●	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Butanoic acid[e,h]	□	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
3-Methyl butanoic acid[e,h]	○	○	●	●	○	○	○	○	○	○	○	○	○	○	○	○	●	○
Pentanoic acid[e,h]	●	●	○	○	○	○	○	○	○	○	○	○	●	○	○	○	●	○
Hexanoic acid[e,f,h]	●	●	●	●	○	○	○	○	○	○	○	○	●	○	○	●	●	●
Heptanoic acid[e,h]	●	●	○	○	○	○	○	○	○	○	○	○	●	○	○	○	●	○
Octanoic acid[e,h]	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	○
Nonanoic acid[e,h]	○	○	●	○	○	○	○	○	○	○	○	○	○	○	○	○	●	○
Decanoic acid	○	○	●	●	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Dodecanoic acid	○	○	●	●	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Tridecanoic acid	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Tetradecanoic acid[e,h]	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Hexadecanoic acid	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
7-Octenoic acid[e,g,h]	○	○	●	●	○	○	○	○	○	○	○	○	○	○	○	●	●	○
3-Methyl-2-hexenoic acid	○	○	●	●	○	○	○	○	○	○	○	○	●	○	○	●	●	○
Alcohols and heterocyclics																		
1-Hexen-3-ol	●	●	●	●	○	○	○	○	○	○	○	○	●	○	○	●	○	○
1-Hepten-3-ol	●	●	●	●	○	○	○	○	○	○	○	○	○	○	○	○	○	○
1-Octen-3-ol	○	○	●	●	○	○	○	○	○	○	○	○	○	○	○	○	○	○
1-Dodecanol	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
3-Methyl-1-butanol[e,i]	●	●	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
2-Phenoxy ethanol		○			○	○	○	○	○	○	○	○	●	○	○	○	○	○
Phenol	●	●	○	○	●	●	○	○	●	●	○	○	●	●	○	●	●	●
2-Methylphenol	●	●					○	○	●	●	○	○	●	●			●	●
4-Methylphenol	●	●	●	●	●	●	○	○	●	●	○	○	●	●	○	●	●	○
4-Ethylphenol[e,i]	●	●	●	●	●	●	○	○	●	●	●	○	●	●	○	●	●	○
Indole[e,i]	○	●	○	○	○	○	○	○	●	●	○	○	●	●	○	○	●	○

Table 1 Continued

Compounds	TE1		TE2		TC1		TC2		TC3		TC4		pTE1		pTE2		pTE3	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
3-Methylindole	○	○	○	○	○	○	○	○	○	○	○	○	●	○	□	○	●	●
Ketones																		
Butanone	○	○	○	○	○	○	○	○	○	○	○	○	○	○	⬤	●	○	○
Geranyl acetone[e,i]	○	○	⬤	⬤	○	○	⬤	○	○	○	○	○	○	○	○	□	○	○
2-Nonanone	○	○	●	●	○	○	○	○	○	○	○	○	○	○	○	□	○	○
6-Methyl-5-hepten-2-one	●	○	○	○	○	○	○	○	○	○	○	○	●	○	○	○	○	○
Esters																		
Methyl propanoate	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Ethyl propanoate	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Others																		
Heptanal	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	○
Heptane	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Dimethyldisulfide	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Water	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Tertyl-butyl methyl ether	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○

All stimuli were tested at 10^{-2} dilution, with the exception of ammonia, which was tested at 2.5%. The criterion for an excitation response was an increase of action potential frequency higher than the SD of the spontaneous activity. The intensity of the responses has been expressed as a percentage relative to the highest response (160 action potentials per second) and is depicted by the symbols: ○, No response; □, inhibition; ●, 0–20%; ●, 20–50%; ⬤, 50–80%; ⬤, >80%. Empty positions: no recording. The number of replicates for each type of sensillum resulting from the cluster analysis were TE1: $n = 15$; TE2: $n = 5$; TC1: $n = 10$; TC2: $n = 6$; TC3: $n = 4$; TC4: $n = 5$; GP1: $n = 8$; GP2: $n = 6$; GP3: $n = 3$; GP4: $n = 2$; GP5: $n = 1$. [a–g]: behavioral activities found for *Anopheles gambiae* in bioassays.
[a]Braks et al. 2001.
[b]Smallegange et al. 2005.
[c]Healy and Copland 2002.
[d]Dekker et al. 2002.
[e]Qiu 2005.
[f]Smallegange et al. 2002.
[g]Costantini et al. 2001.
[h]attraction.
[i]repellence.

between clusters. The classification at the sensillum level was based on the response spectrum of the most responsive neuron in the sensillum, and as a second criterion, the absence or presence of responses to a specific ligand was used. For example, the occurrence of a response to indole in trichoid sensilla C 1 (TC1) classifies it as a sensillum type distinct from trichoid sensilla E 1 (TE1).

Dose–response relationships were analyzed by a general linear model (GLM), Univariate procedure (SPSS for Windows, release 10.0.5). Stimuli and concentration were set as fixed factors. Interactions between the fixed factors were excluded from the model when the interaction effect was not significant. The effect of a fixed factor was tested by the *F*-statistic and was considered to be significant when $P < 0.05$.

Results

Trichoid sensilla and their subtypes and GP can be distinguished using their appearance under the light microscope (Figure 1). Antennal trichoid sensilla E (TE) of *A. gambiae* (Boo 1980) were the shortest trichoid sensilla having a sharp tip and a length of 16.9 ± 1.4 μm (Mean ± SD, Figure 1c). Trichoid sensilla C (TC) had a similar shape as TE but were longer (mean length 21.3 ± 2.2 μm; Figure 1b,c). The visual distinction between TE and TC was in some cases ambiguous; some sensilla have intermediate length and were classified post hoc based on their response spectra.

We recorded the electrophysiological responses of 45 trichoid and 20 GP on segments 6–13 of the antennae of female *A. gambiae* to a panel of 44 odor stimuli (Tables 1 and 2). As

Table 2 Response spectra of each ORN class innervating GP

Compounds	GP1			GP2			GP3			GP4			GP5		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Ammonia and amines															
Ammonia[a,b]	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○
1-Butylamine	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○
1-Pentylamine	●	●	○	●	●	○	○	○	○	●	○	○	●	●	○
Oxocarboxylic acids															
2-Oxobutanoic acid[c,h]	○	○	○	●	●	○	○	○	○	●	●	○	○	○	○
2-Oxopentanoic acid[c,h]	○	○	○	●	●	○	○	○	○	○	○	○	○	○	○
2-Oxohexanoic acid	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Carboxylic acids															
L(+) Lactic acid[a,b,d,h]	○	○	○	○	○	○	●	○	●	●	●	○	○	○	○
Acetic acid	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Propanoic acid[e,h]	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Butanoic acid[e,h]	○	○	○	○	○	○	○	○	○	□	○	○	●	●	○
3-Methyl butanoic acid[e,h]	○	○	○	○	○	○	○	○	○	□	○	○	●	●	○
Pentanoic acid[e,h]	○	○	○	○	○	○	○	○	○	□	○	○	●	●	○
Hexanoic acid[e,f,h]	○	○	○	○	○	○	○	○	○	□	□	○	●	○	○
Heptanoic acid[e,h]	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Octanoic acid[e,h]	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Nonanoic acid[e,h]	○	○	○	○	○	○	○	○	○	□	○	○	○	○	○
Decanoic acid	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Dodecanoic acid	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Tridecanoic acid	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Tetradecanoic acid[e,h]	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Hexadecanoic acid	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
7-Octenoic acid[e,g,h]	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
3-Methyl-2-hexenoic acid	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Alcohols and heterocyclics															
1-Hexen-3-ol	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
1-Hepten-3-ol	○	○	○	○	○	○	○	○	○	□	○	○	○	○	○
1-Octen-3-ol	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
1-Dodecanol	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
3-Methyl-1-butanol[e,i]	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
2-Phenoxy ethanol	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Phenol	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
2-Methylphenol	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
4-Methylphenol	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
4-Ethylphenol[e,i]	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Indole[e,i]	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○

Table 2 Continued

Compounds	GP1			GP2			GP3			GP4			GP5		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
3-Methylindole	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Ketones															
Butanone	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Geranyl acetone[e,i]	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
2-Nonanone	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
6-methyl-5-hepten-2-one	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Esters															
Methyl propanoate	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Ethyl propanoate	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Others															
Heptanal	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Heptane	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Dimethyldisulfide	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Water	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Tertyl-butyl methyl ether	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○

Response level: ○, No response; □, inhibition; ●, 0–20%; ●, 20–50%; ●, 50–80%. See legend of Table 1.

37.2 ms of the total latency (42 ± 12 ms) between the onset of the stimuli and the start of the neuron response can be attributed to the travel time of the stimulus from its point of injection into the airstream to reach the sensillum, the actual response latency was 5.8 ms. This value was similar for trichoid and GP (Mann–Whitney *U*-test, $P = 0.08$). In most cases, spontaneous activity of more than one neuron was recorded (Figure 2). Fourteen compounds as well as the blank controls did not elicit a response from any of the sensilla tested (Table 1).

Functional types of trichoid sensilla

Single-sensillum recordings from TE and TC displayed spontaneous action potentials from 2 ORNs, based on differences in their amplitudes and doublets (Figure 2a–d). We hereafter named the neuron for which we recorded a larger amplitude neuron A and the one with a smaller amplitude neuron B. The levels of spontaneous activity of neurons in TE and TC were similar, the A-neuron (21 ± 8 spikes/s, $n = 45$) showing a higher spontaneous activity than the B-neuron (15 ± 7 spikes/s, $n = 45$) (*t*-test, $P = 0.011$). Most ORNs innervating TE and all ORNs innervating TC responded to the tested odor stimuli by excitation (Figure 2a–d), in a small proportion of TE ORNs inhibition of spontaneous activity was found (Table 1).

Cluster analysis of responses of ORNs in 45 trichoid sensilla to 4 odor stimuli resulted in 6 functional types (Table 1 and Figure 3a–f). As shown in Table 1, 27 out of 44 compounds elicited a response in ORNs in trichoid sensilla. The responses of ORNs to a set of 13 compounds that elicited the highest response intensities are shown in Figure 3. Two functional types were mainly associated with TE (TE1 and TE2). Four functional types were mainly associated with TC and were named TC1-4.

The largest proportion of trichoid sensilla we studied belonged to TE1 (15 out of 45). One or both of the ORNs in TE1 showed excitation responses to 14 stimuli. Both TE1-neurons responded to ammonia, C5-7 carboxylic acids, 1-hexen-3-ol, 3-methyl-1-butanol, phenolics, and indole (Table 1 and Figure 3a). Only TE1A responded to 2-phenoxy ethanol and 6-methyl-5-hepten-2-one by excitation and to C3-C4 carboxylic acids by inhibition. Neuron TE1A showed the strongest responses to 4-methyl- and 4-ethylphenol and intermediate response intensity to 1-hexen-3-ol, indole, and phenol. Ammonia was the strongest stimulant for neuron TE1B, followed by indole and 3 phenolic compounds. TE1A-neurons responded to the majority of stimuli (8 out of 14) more strongly than TE1B-neurons (*t*-test, $P < 0.05$), both neurons responded similarly to the other 6 odors (paired sample *t*-test, $P > 0.05$).

TE2 formed a less abundant functional type (5 out of 45) containing 2 neurons with relatively broad response spectra. TE2-neurons were responsive to 18 of the 40 stimuli tested (Table 1). TE2A responded most strongly to ammonia and

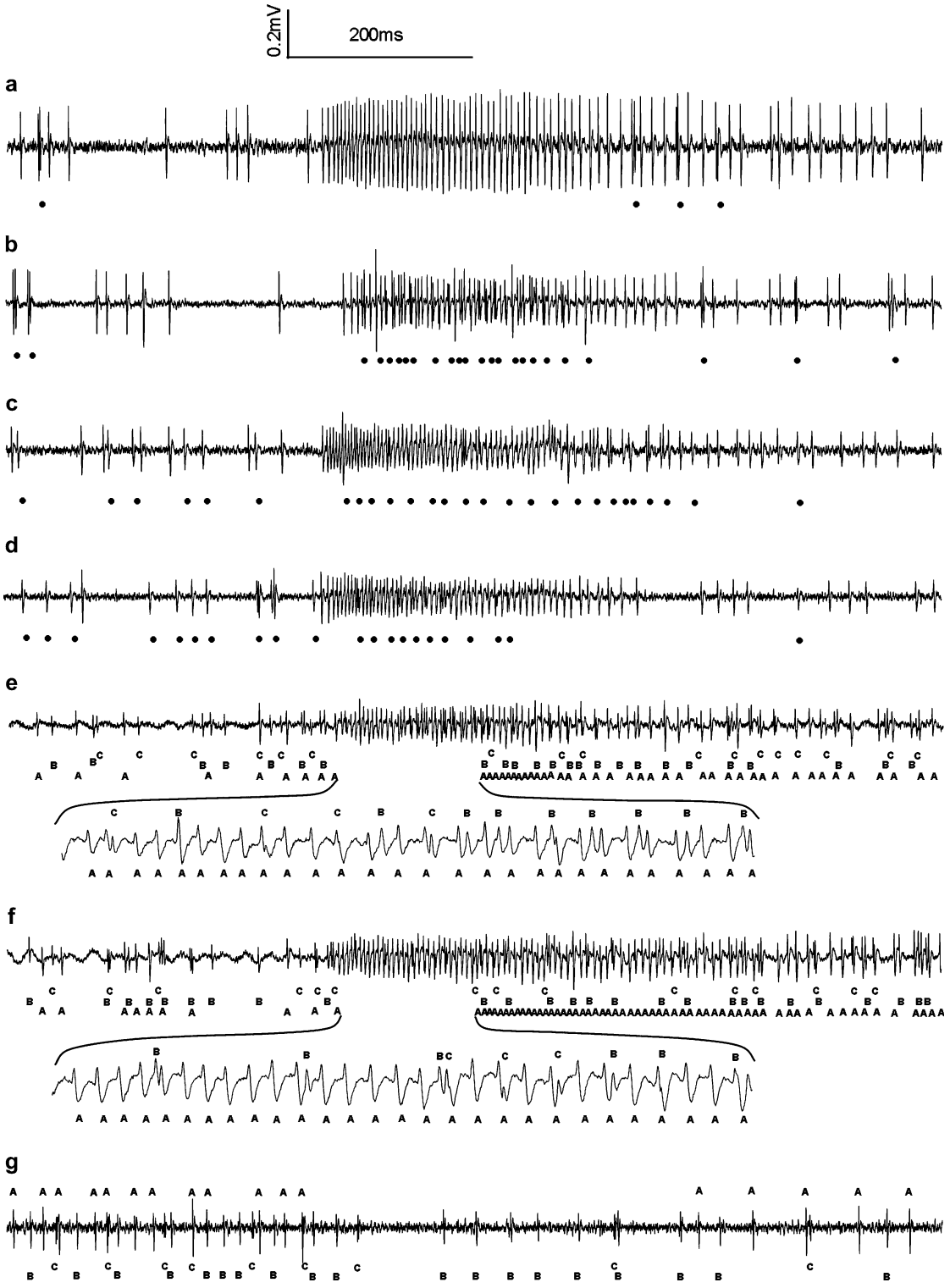

Figure 2 Examples of action potentials from different neurons during the odor stimulation. Horizontal bar indicates the onset and end of odor delivery and vertical bar shows the scale of 0.2 mV. **(a–d)** Black dots indicate the spikes from neuron B, and the other spikes are from neuron A. The spikes from each neuron were marked with A, B, and C in **(e–g)**. Excitation responses include (a) TE1 to 1% 4-ethylphenol, (b) TC1 to 1% 4-methylphenol, (c) TE2 to 1% geranyl acetone, (d) TC2 to 1% geranyl acetone, and (e, f) GP3 to 0.25% ammonia and 1% L-lactic acid. Inhibition response (g) GP4 to 1% 3-methyl butanoic acid.

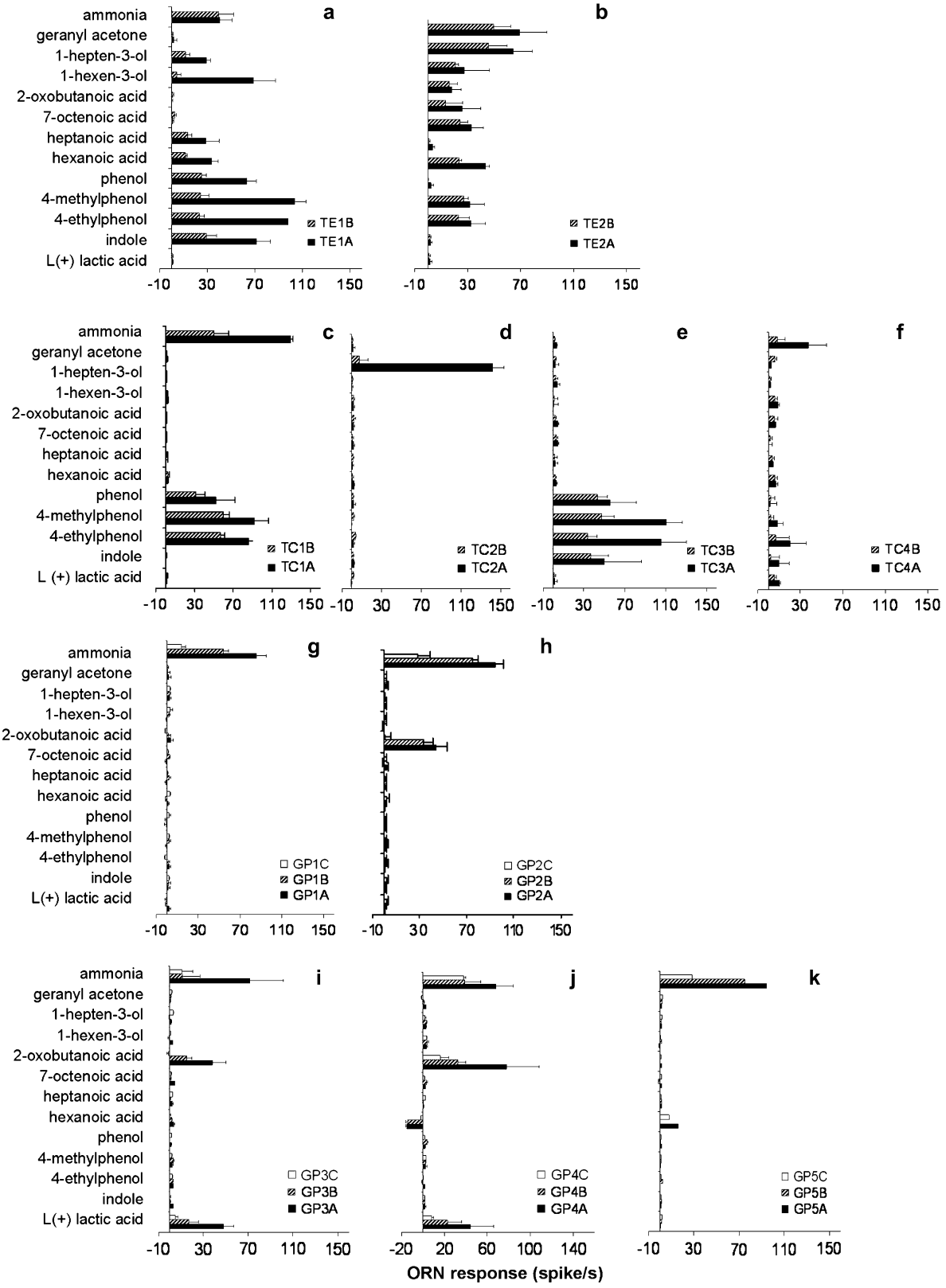

Figure 3 Response patterns of ORNs innervating TE **(a, b)** and C **(c-f)** and GP **(g–k)** of female *Anopheles gambiae* mosquito antennae to 13 odors at a dilution of 10^{-2} (ammonia at 2.5%) listed along the vertical axis of the bar graphs. Solid bars: neuron A, hatched bar: neuron B, and empty bar: neuron C. For number of replicates for each group see Table 1. Error bars are standard error of mean.

geranyl acetone, whereas hexanoic acid, 4-methyl- and 4-ethylphenol, and 7-octenoic acid were weaker stimulants (Figure 3b). TE2A also showed responses to propanoic acid, 3-methyl butanoic acid, C9-10 and C12 carboxylic acids, 3-methyl-2-hexenoic acid, 1-hexen-3-ol, 1-hepten-3-ol, and 2-nonanone. TE2B-neurons showed response spectra and sensitivity similar to those of TE2A (paired sample *t*-test, $P > 0.05$) except that they were not responsive to nonanoic acid and had a weaker response to hexanoic acid than TE2A (*t*-test, $P < 0.05$).

Functional type TC1 was relatively common (10 out of 45). TC1A- and TC1B-neurons were responsive to phenols and ammonia; response intensities of these neurons were similar (paired sample *t*-test, $P > 0.05$) (Table 1 and Figure 3c). The most specific functional type we found was TC2 (6 out of 45), in which only TC2A was responsive to geranyl acetone (Table 1 and Figure 3d). A small proportion of trichoid sensilla belonged to functional type TC3 (4 out of 45). This functional type contained 2 ORNs responsive to phenols and indole (Table 1 and Figure 3e). Another distinct functional type was TC4 (5 out of 45), of which TC4A-neurons responded to ammonia and 4-ethylphenol and TC4B was unresponsive to any of the test stimuli (Table 1 and Figure 3f).

Functional types of GP

Single-sensillum recordings from GP (Figure 2e–g) exhibited electrophysiological activity of 3 neurons. Neurons with large, medium, and small spike amplitudes have been designated as A, B, and C, respectively (Figure 2e,f). All sensilla we recorded from contained at least one neuron that responded to ammonia and 1-butylamine. Cluster analysis revealed at least 5 functional types that could be identified among 20 GP according to their responses to L-lactic acid, 2-oxobutanoic acid, and hexanoic acid. The spontaneous activity of neurons in different functional types of GP was similar, the A-neuron exhibiting the highest (45 ± 16 spikes/s, $n = 20$), the B-neuron intermediate (33 ± 14 spikes/s, $n = 20$), and the C-neuron the lowest spontaneous activity (15 ± 12 spikes/s, $n = 20$) (analysis of variance, $P < 0.05$). Functional type grooved-peg sensilla 1 (GP1) appeared to be most abundant (8 out of 20). ORNs in this type responded to ammonia but not to L-lactic acid, 2-oxobutanoic acid, and hexanoic acid (Table 2 and Figure 3g). ORNs in functional type GP2, to which 3 out of 20 sensilla belonged, were tuned to 2-oxobutanoic acid in addition to ammonia (Table 2 and Figure 3h). ORNs in functional type GP3 (6 out of 20 sensilla tested) were tuned to L-lactic acid in addition to ammonia and 2-oxobutanoic acid (Table 2 and Figure 3i). GP4-neurons (2 out of 20 sensilla tested) displayed excitation responses to ammonia, L-lactic acid, and 2-oxobutanoic acid and were inhibited by C4-C6 and C9 carboxylic acids (Table 2 and Figure 3j). One sensillum type, GP5, contained ORNs responding to C5-C6 carboxylic acids by excitation (Table 2 and Figure 3k).

Dose–response relationships

The dose–response relationships of neurons innervating 3 functional types of trichoid sensillum TE1, TC1, and TC3 to 4-ethylphenol are shown in Figure 4a. A-neurons in all 3 functional types responded more intensely to 4-ethylphenol than B-neurons, and the sensitivity of the A- or B-neurons to 4-ethylphenol in the 3 types was similar (GLM). The dose–response relationship of these neurons for 4-methylphenol was similar to that for 4-ethylphenol (Figure 4b). The dose–response curves of TE2- and TC2-neurons to geranyl acetone are shown in Figure 4c. The A-neurons in these 2 sensillum types had similar sensitivity to geranyl acetone, whereas neuron TE2B showed much higher sensitivity than neuron TC2B. The dose–response relationships for ammonia recorded from neurons TE1A, TE1B, and TC4A were similar and revealed a higher sensitivity than found in neuron TC4B (Figure 4d).

Figure 4e–f illustrates dose–response curves of TE1A-neurons to their most effective stimulants. This neuron type had the lowest threshold to indole but the responses saturated above the 10^{-3} dilution. The responses of neuron TE1A to the 10^{-3} dilution of phenols were lower than to indole but increased linearly with concentration (Figure 4e). The sensitivity to 1-hexen-3-ol was higher than to 1-hepten-3-ol (Figure 4f). The dose–response curves to hexanoic acid were comparable with that of 1-hepten-3-ol and ammonia.

The sensitivities of neurons innervating GP to ammonia, L-lactic acid, and 2-oxobutanoic acid are shown in Figure 5. In both GP1 and GP3 sensilla, the A-neuron had the highest and the C-neuron the lowest sensitivity to ammonia (Figure 5a,b). For neurons GP1A and GP3A, the threshold concentration was below the 10^{-5} dilution. Neurons A and B in GP3 were responsive to L-lactic acid; GP3C was not responsive to any concentration tested (Figure 5c). Neurons GP3A and GP3B displayed a similar sensitivity to 2-oxobutanoic acid, whereas that of neuron GP3C was lower (Figure 5d).

Effect of blood feeding on ORN responses

In female mosquitoes that had taken a blood meal, 3 functional groups of TE, namely, pTE1, pTE2, and pTE3, were identified (Table 1, "p" for postblood feeding). Eight out of 16 of the sensilla studied belong to pTE1 (Table 1); ORNs in these sensilla had a similar response spectrum as those in TE1 (Figures 3a and 6a). One or both of the ORNs of pTE1 responded by excitation to 14 stimuli. The responses of pTE1A to these compounds were not different from TE1A except for phenol (*t*-test, $P = 0.032$), to which pTE1A had a higher response. In contrast to TE1A, pTE1A was not excited by hepten-3-ol and 3-methyl-1-butanol and not inhibited by C3-4 carboxylic acids. However, pTE1A was excited by 3-methyl-2-hexenoic acid and 3-methylindole, which did not excite TE1A-neurons (Table 1). The B-neuron in the pTE1 sensillum was less responsive to stimulation than TE1B. As shown in Table 1 and Figure 6a, this neuron

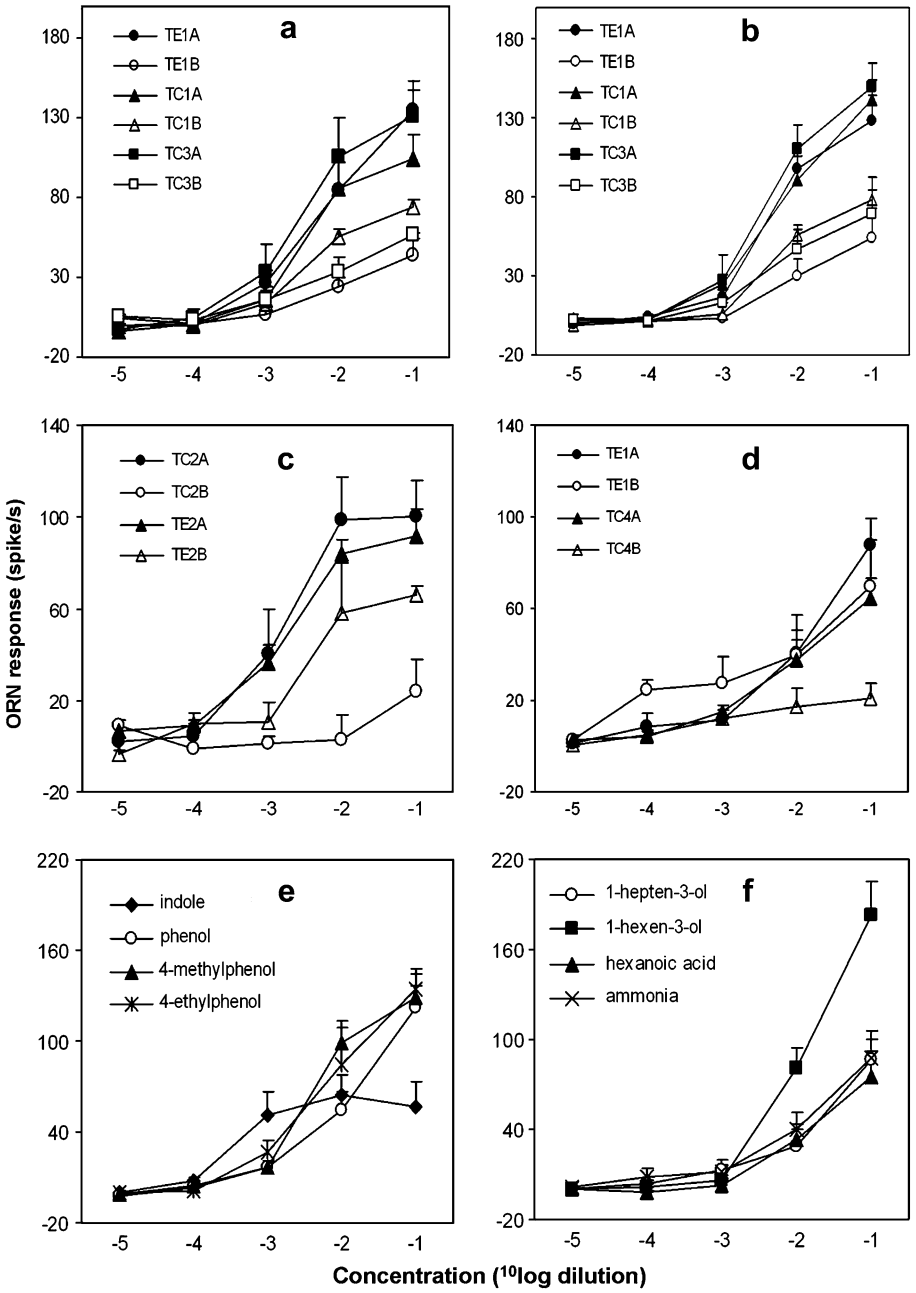

Figure 4 Dose–response curves of ORNs to odor stimuli at 5 concentrations. The *x* axis shows the logarithms of the dilutions. **(a–d)** shows dose–response curves of different neurons to the same odorant stimuli. (a) neurons in TE1, TC1, and TC3 (n = 5, 6, and 3) to 4-ethylphenol; (b) neurons in TE1, TC1, and TC3 (n = 5, 6, and 3) to 4-methylphenol; (c) neurons in TC2 and TE2 (n = 4 and 2) to geranyl acetone; (d) neurons in TE1 (n = 5) and TC4 (n = 5) to ammonia. **(e, f)** represent neurons in TE1 responding to different odor stimuli (n = 5). Error bars are standard error of mean.

was weakly responsive to ammonia, phenols, and indole but not to other compounds. Unlike TE1B, neuron pTE1B was not responsive to C5-7 carboxylic acids, 1-hexen-3-ol, 1-hepten-3-ol, and 3-methyl-1-butanol.

Before blood feeding, TE2 contains ORNs responsive to geranyl acetone. We found a functional type, pTE2, in mosquitoes after blood feeding, which contained geranyl acetone–sensitive neurons. Four out of 16 TE tested belonged to this type. There was overlap of response spectra between this functional type and TE2, for example, both types contained ORNs responsive to ammonia, hexanoic acid, 7-octenoic acid, 3-methyl-2-hexenoic acid, 1-hexen-3-ol, 1-octen-3-ol, phenols, and geranyl acetone. A substantial difference between TE2 and pTE2 was the clear differentiation of neurons A and B in pTE2. Unlike neurons TE2A and TE2B, which had similar response spectra, pTE2A and

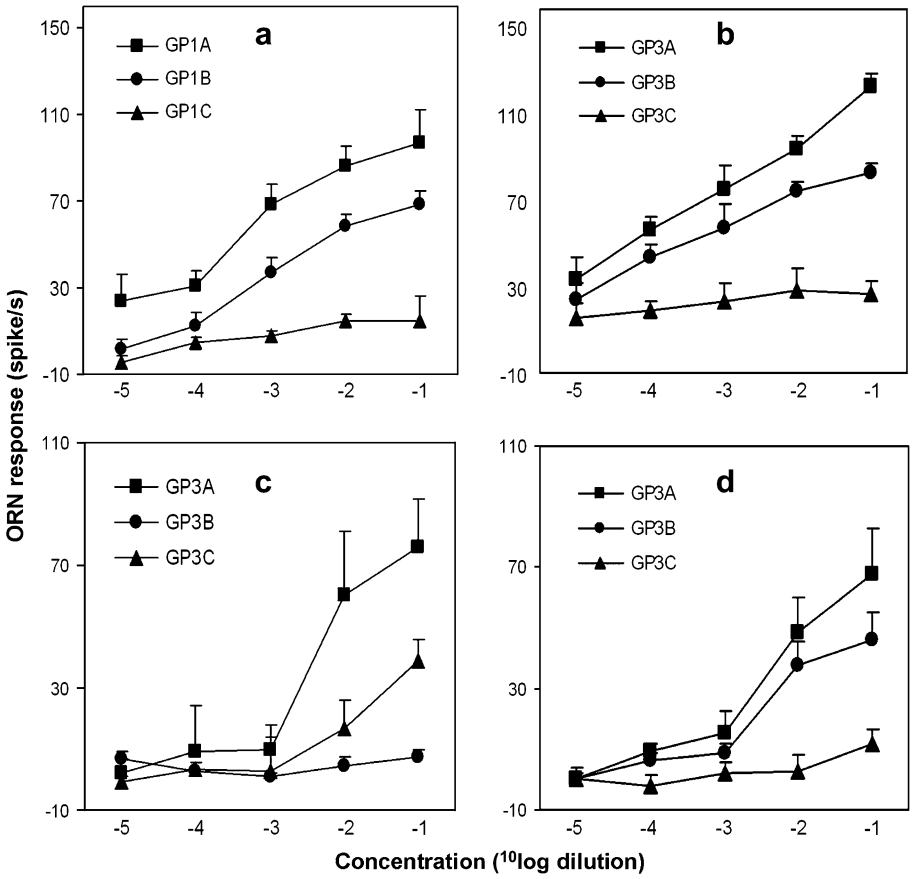

Figure 5 Dose–response curves of ORNs in GP to odor stimuli. The *x* axis shows the logarithm of the dilutions (for ammonia, 10^{-1} dilution corresponds with 2.5%). **(a, b)** GP1- and GP3-neurons responding to ammonia, **(c)** GP3-neurons to L-lactic acid, **(d)** GP3-neurons to 2-oxobutanoic acid. Error bars are standard error of mean.

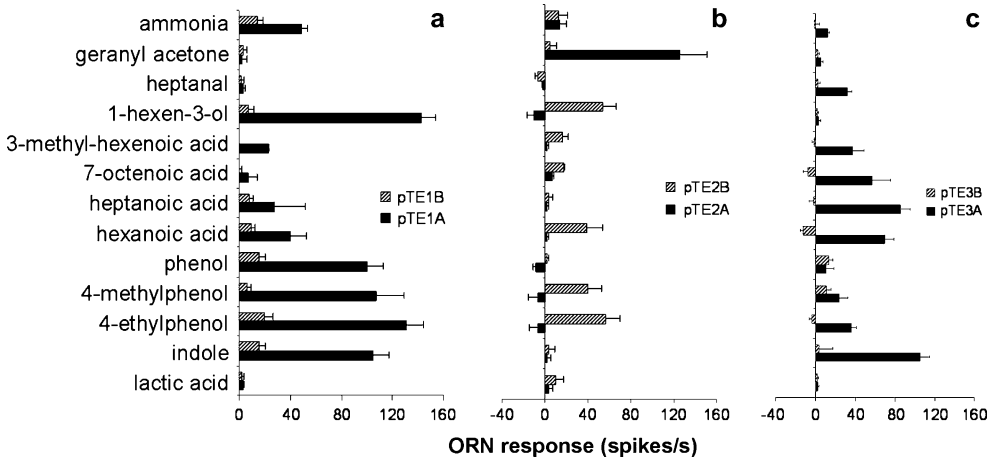

Figure 6 Response patterns of ORNs innervating trichoid sensilla and GP on female *Anopheles gambiae* mosquito antennae to 13 odors at a dilution of 10^{-2} listed along the vertical axis of the bar graphs. Solid bars: neuron A, hatched bar: neuron B. **(a)** pTE1, **(b)** pTE2, **(c)** pTE3. For number of replicates for different sensilla see Table 1. Error bars are standard error of mean.

pTE2B had different response spectra: neuron pTE2A was specifically tuned to geranyl acetone, whereas neuron pTE2B was tuned to a broader range of compounds (Table 1 and Figures 1b and 6b).

A third functional type of TE, pTE3, was found only after blood feeding. Four out of 16 TE belonged to this type. Although this type contains one neuron tuned to indole, the overall pattern of response spectra was dissimilar to the

indole-sensitive TE1-neurons (Table 1 and Figure 6c). Neurons in pTE3 displayed a higher total spontaneous action potential activity (37 ± 8 spikes/s) than the neurons in pTE1 (16 ± 7 spikes/s) (*t*-test, $P < 0.001$). Neuron pTE3A responded by excitation to 17 compounds (Table 1). This neuron responded most strongly to indole, followed by C7-8 carboxylic acids. Neuron pTE3A was the only one that responded to heptanal among all the neurons we studied before or after a blood meal. The 2 human axillary odors, 7-octenoic acid and 3-methyl-2-hexenoic acid, elicited excitation responses in neuron pTE3A. Neuron pTE3B was less responsive than pTE3A; it responded only weakly to hexanoic acid, phenols, and 3-methylindole.

When compared with neuron TE1A, the sensitivity of neuron pTE3A to C6-9 carboxylic acids was higher than of neuron TE1A, whereas TE1A had higher sensitivities to 3 phenols than pTE3A (Figure 7a,b). Neuron pTE3A was more sensitive to indole than TE1A and pTE1A at lower doses (dilution 10^{-4} to 10^{-6}); the response decreased at the 2 highest doses (Figure 7c). A GLM analysis showed that neuron pTE3A was most sensitive to indole, pTE1A was intermediate, and TE1A was the least sensitive.

GP contain neurons sensitive to ammonia both before and after blood feeding. The response thresholds of the 2 most responsive neurons pGP1A and pGP1B were higher after blood feeding (Figure 7d). Neuron pGP1B was less sensitive to ammonia after blood feeding than GP1B (GLM, $P < 0.05$).

Temporal aspects of ORN responses

The temporal patterns of ORN activity depended on the odor stimulus. The predominant temporal response pattern is phasic–tonic, whereas a tonic pattern was occasionally found in TE. As shown in Figure 8, the excitation response of TE1A showed prolonged responses to phenols (Figure 8a,e), which persisted beyond the end of stimulus delivery. Responses of this neuron to indole lasted for a shorter period than to phenols (Figure 8b,f). In contrast, the same neuron showed excitation responses to 1-hexen-3-ol (Figure 8c,g) and 3-methyl-1-butenol (not shown) that stopped abruptly when stimulus delivery ended (poststimulus quiescence). However, when stimulated with higher concentrations of 1-hexen-3-ol, a prolonged response pattern was produced (Figure 8d,h).

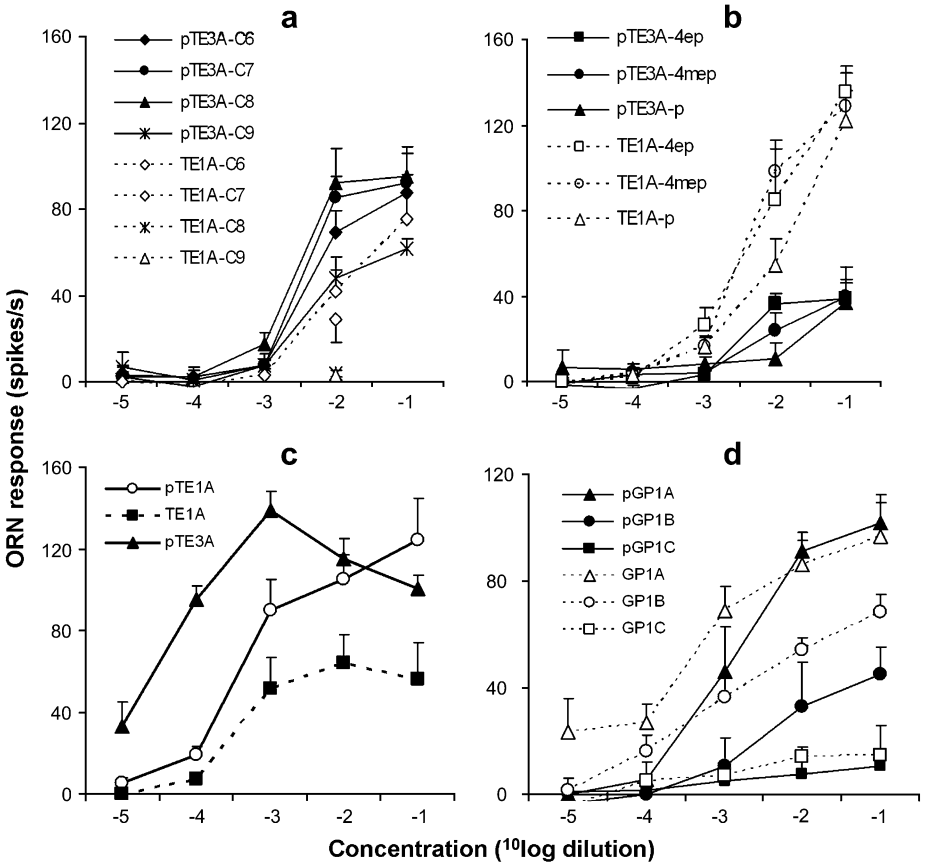

Figure 7 Dose–response curves of ORNs to odor stimuli at 5 concentrations. The *x* axis shows the logarithms of the dilutions. **(a)** responses of pTE3A and TE1A to C6-9 carboxylic acids; **(b)** responses of pTE3A and TE1A to 3 phenols, "4ep," 4-ethylphenol, "4mep," 4-methylphenol, and "p," phenol; **(c)** responses of pTE1A, pTE3A, and TE1A to indole; **(d)** responses of pGP1A and GP1A to ammonia. Error bars are standard error of mean.

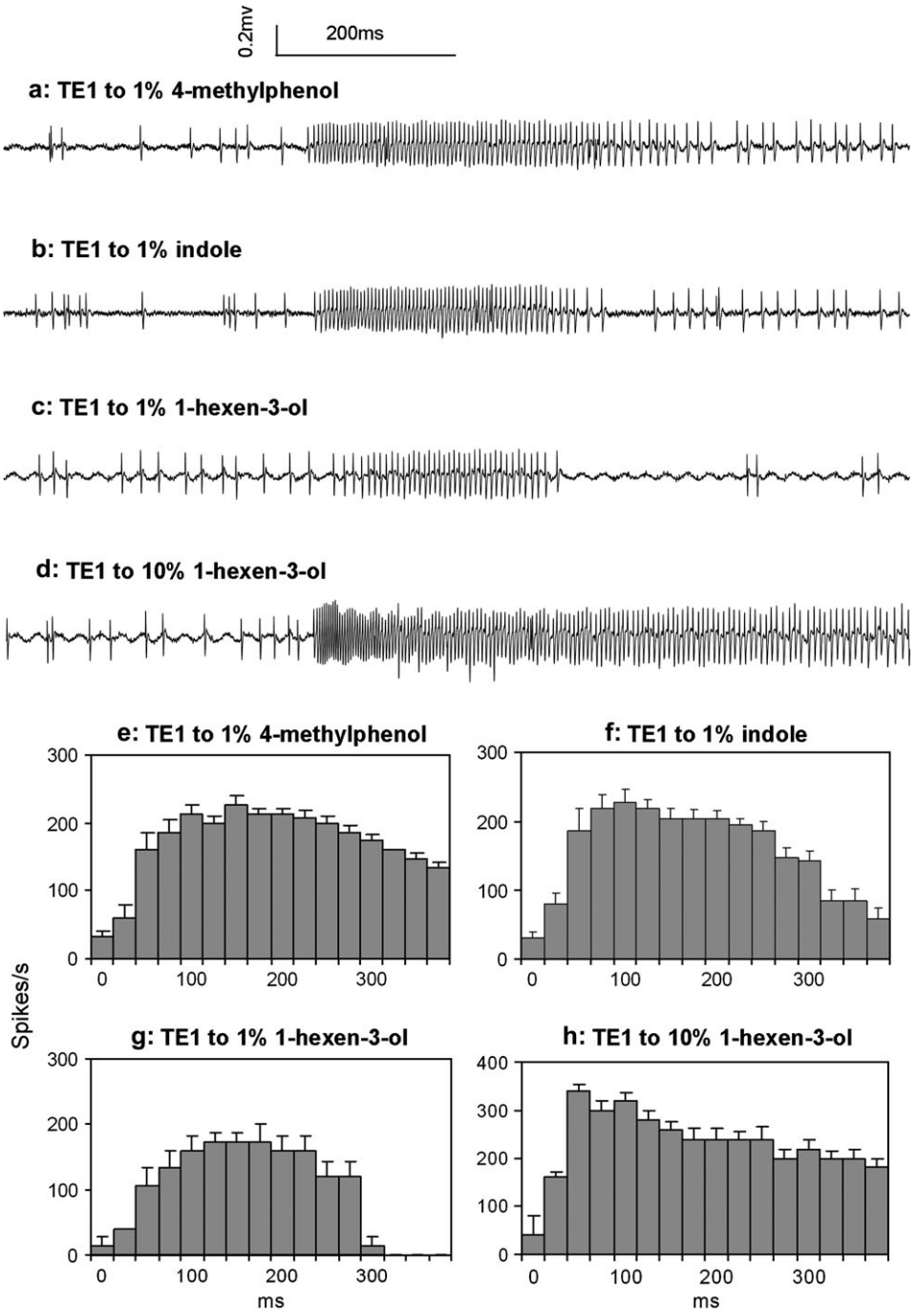

Figure 8 Temporal characteristics of ORNs in trichoid sensilla TE1 in response to different odor stimuli. Horizontal bar indicates the onset and end of the stimulations and vertical bar shows the voltage scale (0.2 mV). The spike trains **(a–d)** have all been recorded from the same sensillum in response to the stimuli indicated. **(e)** histogram of action potential frequency over time of TE1 responding to 1% 4-methylphenol, showing a typical prolonged phasic–tonic response ($n = 6$). **(f)** histogram showing abrupt decrease of the firing frequency shortly after the end of the stimulus ($n = 9$). **(g)** frequency histogram of TE1 responding to 1-hexen-3-ol, showing a clear phasic character ($n = 3$); the response to 1-hexen-3-ol changed to a phasic–tonic firing mode at a higher concentration (10%) **(h**, $n = 2$). Gray bars in (e–h) are mean frequency (Hz) in intervals of 25 ms during the first 400 ms after the onset of the odor stimuli, error bars are standard error of mean.

Distribution map of functional sensillum types on the antennae

Approximately 30 TE, 42 TC, and 59 GP were located on the 6th to 13th segment of a female mosquito antenna (Figure 9a). A topical map was constructed of the 6 functional types of trichoid and 5 types of GP on the antennae of female mosquitoes (Figure 9b). More than half of the recordings were made from sensilla located on segments 12 and 13. We saw

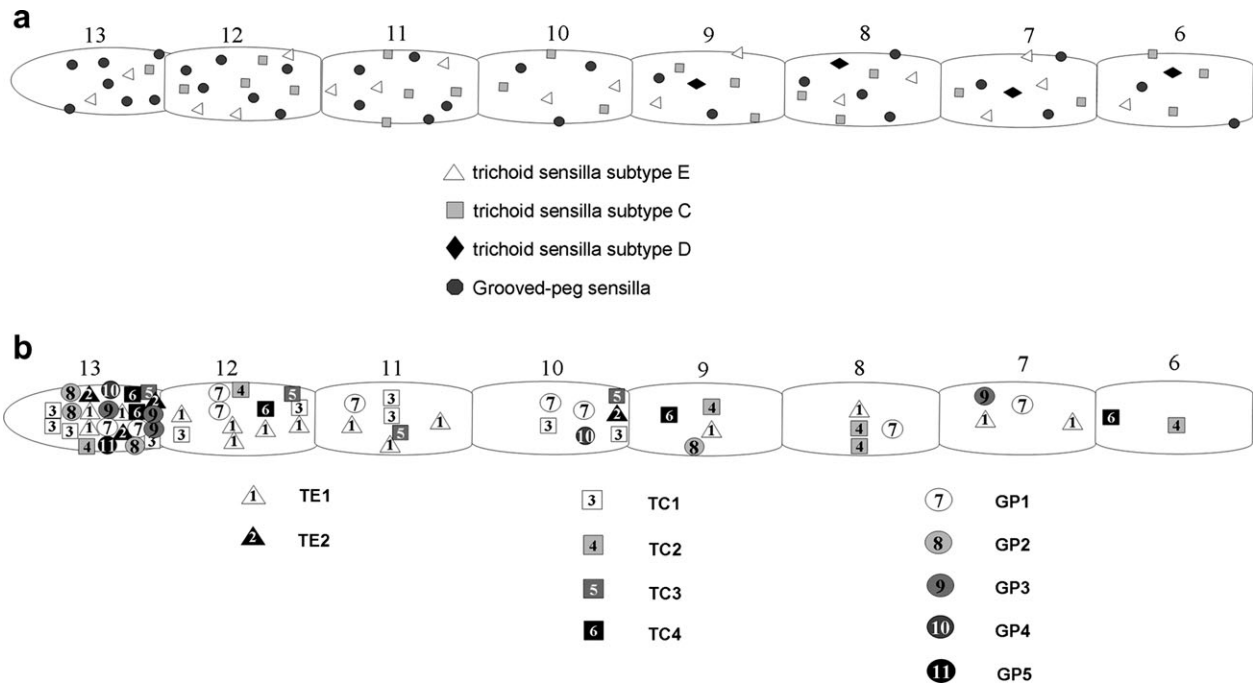

Figure 9 **(a)** A map of various types of olfactory sensilla located on the 6th to 13th antennomeres of female *Anopheles gambiae*, showing one side of the segment. The number of each type was based on half of the average number from 7 antennae observed from the dorsal side. **(b)** a functional map of 6 types of trichoid sensilla and 5 types of GP according to the in vivo position when the recordings were made. The position along the longitudinal axis was more reliable than along the circumference due to possible twisting of the antenna.

no evidence of a distinct distribution pattern of different functional sensilla types across antennal segments.

Discussion

Role of functional neuron classes in odor coding

We present here the first systematic study on the function of olfactory neurons in 3 types of antennal sensilla of *A. gambiae*. Six functional types of trichoid and 5 functional types of GP were identified based on their response spectra to 5 and 3 odor stimuli, respectively. ''Generalist'' ORNs that are tuned to a broad range of odors were found in trichoid sensilla subtype E, whereas ''moderate specialist'' ORNs that are tuned to a narrow range of odors were found in subtype C and GP. In the latter 2 sensillum types, 2 ''extreme specialists'' were identified that responded to only one odor out of the 44 tested. Response spectra of trichoid E and C and GP overlapped. No overlap was found between response spectra of trichoid C and GP except for ammonia. Ammonia is thus far the only compound documented to attract *A. gambiae* on its own (Smallegange et al. 2005). The fact that ORNs sensitive to ammonia in the majority of sensilla cooccur with neurons with different specificity spectra allows the insect to accurately measure the ratio between ammonia and other ligands, which might contain information on the producer of the blend (Barata et al. 2002).

Neurons cocompartmentalized in the same sensillum overall exhibited similar response spectra, but their sensitivity differed. Similar specificity but different sensitivity (Figure 4a–d) might help the mosquito to increase the resolution of concentrations encountered in flight. Our findings are in agreement with those previously reported for *A. gambiae* (Van Den Broek and Den Otter 1999; Meijerink et al. 2001).

ORNs are believed to be tuned to a limited number of odors that are important for survival and reproduction of the organism. We here report that only in 11% of the neuron–stimuli combinations studied, responses were elicited (Table 1). This proportion is comparable with the 15% found for *Drosophila* (De Bruyne et al. 2001). Three of the 27 identified neuron types did not respond to any of the test odors suggesting that the odors to which these neurons are tuned were not included in our test set.

In this study, we identified 6 functional types of sensilla trichodea, each containing 2 responding ORNs, and 5 functional types of GP, each containing 3 responding ORNs. At the single-neuron level, neurons in sensilla trichodea E were clearly responding to a wider range (more than 10 stimuli in all cases) than sensilla trichodea C and GP (Table 3). Olfactory neurons that respond to a number of odor molecules with different chemical structures are common in insects (De Bruyne et al. 1999, 2001; Shields and Hildebrand 2001). The paradigm has been that one olfactory neuron expresses only one odorant receptor gene as a 7 transmembrane

Table 3 Percentage of ORNs in sensilla trichodea and GP responding to different numbers of stimuli out of 44 compounds tested

Number of compounds eliciting responses	Sensilla trichodea E (total neuron types = 4)	Sensilla trichodea C (total neuron types = 8)	GP (total neuron types = 15)
0	0	25	7
1–2	0	25	20
3–5	0	50	53
6–10	0	0	13
>10	100	0	7

receptor molecule, but recently evidence was published that 2 genes can be coexpressed in the same ORN (Goldman et al. 2005). By expressing single OR genes in a mutant olfactory neuron, it has been established that one odorant receptor can interact with multiple odorants (Hallem, Ho, and Carlson 2004).

Some compounds caused responses in several different classes of ORNs, ammonia being a particularly clear example. Thus far, no gene of the OR gene family has been found that mediates chemoreception of ammonia (Yao et al. 2005). Overlap in response spectra is considered very important in the odor discrimination performed by the brain (Dethier 1972; Boeckh and Ernst 1983). This overlap was also found in optical imaging studies in the antennal lobe, in which different odors may activate partly the same glomeruli but each activates a unique ensemble of glomeruli (Hansson et al. 2003; Meijerink et al. 2003; Sachse and Galizia 2003). It is assumed that each neuron class sends its axons to one glomerulus. The differences in specificity of ORNs for compounds with similar structures are in accordance with these findings and likely enable the brain to distinguish between odors. In *Drosophila*, different classes of ORNs show little overlap for the same "best" odor stimuli, which is in contrast to our findings in *A. gambiae* (De Bruyne et al. 2001). This contrast might be explained by the contrast in food specialization. *Drosophila* is a generalist feeding on decaying substrates containing yeast, whereas *A. gambiae* and the similarly anthropophilic mosquito *Ae. aegypti* are carnivorous specialists, both preferring humans to other animals for a blood meal (Mciver 1968; White 1974; Mukwaya 1976).

Our findings suggest that across-fiber patterning, more recently also termed combinatorial coding, is a plausible odor coding mechanism operating in female *A. gambiae*: each odor was a ligand for more than one neuron and/or the majority of neurons had more than one ligand. In other words, none of the odors was the only ligand to only one ORN. Although in some insect species indications for labeled-line olfactory coding for host-related odors have been found (Anton and Hansson 1995; Roche-King et al. 2000), across-fiber patterning is considered to be most common (Dethier 1976; Shepherd 1985). We observed that one ORN may respond to different odorants with different temporal characteristics, a phenomenon also reported for *Drosophila* (de Bruyne et al. 2001). Temporal characteristics of ORN responses provide the brain with information on odor plume structure. Bursts of action potentials result into upwind flight (Kaissling 1986; Baker et al. 1988; Baker 1990).

We found that ORNs that were tuned to phenols were always responsive to all the phenols tested, although in a different degree depending on the length and position of the aliphatic side group. This is in disagreement with the high specificity as found by Hallem, Ho, and Carlson (2004) in *Drosophila*. Neurons engineered with the odor receptor gene *AgOr1*, which was found in the antennae of female *A. gambiae* mosquitoes only and was downregulated after a blood meal (Fox et al. 2001), were specifically responsive to 4-methylphenol (Hallem, Fox, et al. 2004). The gene *AgOr2* was shown to confer a specific response to 2-methylphenol. It is possible that OR genes other than *AgOr1* and *AgOr2* are expressed in the phenol-sensitive ORNs we studied. Another explanation might be that the specificity of odorant-binding proteins (OBPs) that occur in the receptor lymph and transport and deliver odor molecules to the receptor molecule in the neuronal membrane had different specificity in *Drosophila* compared with analogous OBPs in *A. gambiae*.

GP had response spectra distinctly different from trichoid sensilla, although overlap was found. These 2 types of sensilla differ fundamentally in their cuticular wall structure (Steinbrecht 1997). Our results support the hypothesis that neurons in GP were tuned to the most polar compounds (Table 2). The difference we found in response spectra between these 2 types of sensilla is in line with the fact that neurons from trichoid sensilla and GP project each into 2 distinct nonoverlapping zones in the antennal lobe (Anton and Hansson 1994).

ORN physiology, behavioral responses, and phenotypic plasticity

Fourteen of the 44 compounds that were tested in the present study were previously shown to exert an attractant effect on *A. gambiae* (Table 1) (Takken et al. 1997; Braks et al. 2001; Costantini et al. 2001; Healy et al. 2002; Smallegange et al. 2002; Qiu 2005; Smallegange et al. 2005). Olfactory neurons tuned to 12 out of these 14 compounds were found in the sensilla types we investigated. We recently demonstrated a tripartite synergistic effect among ammonia, L-lactic acid, and a mixture of 12 carboxylic acids as attractants for *A. gambiae* females (Smallegange et al. 2005). Synergistic effects at the level of ORN responses are known in insects, especially between sex pheromones and plant odors (Dickens et al. 1993; Nikonov and Leal 2002; Ochieng et al. 2002; Said et al. 2005).

Blood feeding induced both up- and downregulation of ORN responsiveness to putative kairomones of female *A. gambiae*. The overall sensitivity to ammonia and phenols

was lower after a blood meal, supporting a role in the observed suppression of host-seeking behavior. Female-specific putative OR genes *AgOr*1 and *AgOr*2 were downregulated after a blood meal (Fox et al. 2001). The overall decrease of sensitivity to phenols we found might be caused by the downregulation of these and similar genes. A recent microarray analysis of 14,900 genes of *A. gambiae* showed 7 antennal proteins with putative olfactory function and 2 OBPs were downregulated following a blood meal whereas one OBP was upregulated (Marinotti et al. 2005). ORN sensitivity changes might also be caused by neuromodulators produced as a result of the blood meal as has been previously suggested for L-lactic acid–sensitive ORNs in *Ae. aegypti* (Davis 1984).

Blood feeding results in the occurrence of a new functional type of trichoid sensilla, pTE3. The total number of TE on the 6th to 13th segment of each antenna is about 30, and we examined 20 of them in mosquitoes that had not taken a blood meal (60%) and found 2 functional types. In mosquitoes not yet having had a blood meal, we never found pTE3. After a blood meal, we found that of TE, 13% were of type pTE3. These numbers must be accompanied by 2 cautionary notes. First, the distinction between TE and TC is in some cases ambiguous, which might cause deviations from the actual proportions in which they occur. Second, as a result of uncontrollable torsion in the antennal shaft during preparing and fixing the mosquito on the substrate, the viewing angle of the antennal segments varies. This may have resulted in different composition of sensillar samples and uneven representation of different functional types.

In view of the timing of occurrence of pTE3 within the gonotrophic cycle, this type might mediate orientation to oviposition sites as these are known to release indoles, phenols, and carboxylic acids (Bentley et al. 1979; Kyorku et al. 1990; Millar et al., 1994). Several mosquito species, including *A. gambiae* s.s., are known to respond to these compounds at the time of oviposition (Allan and Kline 1998; Blackwell and Johnson 2000; Sumba et al. 2004), and sensitivity to indoles and phenols as found in our study appears in agreement with these behavioral findings. These novel findings suggest that some OR genes are only expressed after the first blood meal has been engorged and stresses the need to study the olfactory system of insects in different physiological states.

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