Supporting Information

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SI Materials and Methods

SEM. Female mosquito heads were fixated in acetone for 4 days and then subjected to critical point drying on Denton vacuum desk II. Heads were coated with gold for 120 s (deposits, ≈40 nm) in a Tousimis samdri-780A gold sputter and later observed under a Hitachi S3500N SEM.

Insects. Laboratory reared *Culex quinquefasciatus* were used throughout this study. Details have been described previously (1, 2). For electrophysiology, we used host seeking adults from the colony that were either blood fed and had laid eggs or only sugar fed. Adults were maintained at high humidity and 12 h light/12 h dark photoperiod.

GC Linked Electroantennographic Detection (EAD). GC-EAD was performed on a Hewlett-Packard 5890 Series II plus GC equipped with an HP-5MS column (30 m × 0.25 mm; 0.25 μm; Agilent Technologies) and connected with the transfer line and temperature control unit from Syntech. The temperature program started at 50 °C for 1 min, increased to 250 °C at a rate of 10 °C per minute. The GC was operated under splitless mode with the injection port at 250 °C and a post run at 290 °C for 10 min. Synthetic mixtures were also analyzed with a faster program starting at 80 °C for 1 min, increased to 290 °C at a rate of 15 °C per minute. Effluent from the capillary column was split in to EAD and flame ionization detector (FID) in 3:1 ratio. A host seeking adult *Cx. quinquefasciatus* female was pushed into a truncated pipette tip and secured by modeling clay letting only the eyes and antenna exposed. To prevent the mosquito from crawling out backwards and to provide humidity, we placed a water soaked tissue paper roll in the large end touching the posterior end of the mosquito. The preparation was mounted on a Syntech EAG platform equipped with micromanipulator-12 and a high-impedance AC/DC amplifier (Syntech). Chloridized silver wires in drawn-out glass capillaries filled with 0.1% KCl and 0.5% polyvinylpyrrolidone (PVP) were used for reference and recording electrodes. The reference electrode was placed in the eye, whereas the recording electrode accommodated the two antennae of the restrained mosquito after their tip was clipped to provide a better contact (Fig. 3*A*). Antennal signals were fed into Syntech AMS-01 amplifiers. The analog signals were fed into A/D 35900E interface (Agilent) and acquired simultaneously with a FID signal on an Agilent Chemstation.

Other Chemical Analysis. GC-MS was performed on a 6890 Series GC and a 5973 Network Mass Selective Detector (Agilent Technologies) operated under electron impact (EI) mode. GC was equipped with a HP-5MS capillary column (see above) and operated as in GC-EAD. Identity of each compound was verified by matching elution temperatures and mass spectrum with a synthetic standard. Identity of all biologically active peaks was verified by GC-EAD using synthetic standards.

Single Sensillum Recordings. Seven hundred and six recordings from various sensilla types were made as described previously (1, 2). Up to 10 sensilla were recorded from an individual mosquito. In brief, a mosquito was immobilized by removing wings and legs and was fixed on a square (7.62 × 7.62 cm) platform made of five 7.62 × 2.54 cm × 1-mm microscopic glass slide (Platinum). Antennae were extended smoothly onto the slide covered with double-sided sticky tape. A glass reference was placed in the eye and recording electrode was brought into contact with the sensillum under the microscope (Olympus BX51WI; 800× magnification). Recorded extra cellular action potentials were amplified, digitized and recorded on the hard disk of a PC. Hardware and Software to record and analyze the data were from Syntech. The activity of colocated olfactory receptor neurons (ORNs) in a given sensillum was assessed based on the differences in spike amplitude. The ORN with the largest spike amplitude was named A; whereas the second largest and the smallest were termed as B and C, respectively. Signals were recorded for 10 s (unless otherwise mentioned) starting 2 s before stimulation, and action potentials were counted off-line in a 500-ms period before and during stimulation. Change in spike rates during 500-ms stimulation was subtracted from the spontaneous activity of preceding 500 ms, and counts were converted to the conventional scale of spikes/s.

Stimulation and Stimuli. Stimulation method and protocol were previously described (1, 2). Throughout this study, compressed purified air (zero grade) was used (Airgas) for the main airflow and stimulus delivery. After high responses, a gap of at least 1 min between stimulations was allowed. Once contact was established each sensillum was subjected to 13 mixtures of synthetic chemicals (102 chemicals most are implicated in sensory physiology of mosquitoes and other blood feeding insects). Each sensillum was confronted with these mixtures. If any mixture elicited response, each constituent was tested individually. Various mixtures were prepared based on the functional groups. Alcohols: propanol, butanol, pentanol, hexanol, heptanol, octanol, nonanol, decanol, 3-methyl-1-butanol, 2-butoxyethanol, 4-methylcyclohexanol, 3-octanol, (*Z*)-3-hexene-1-ol, (*E*)-3-hexen-1-ol, (*E*)-2-hexen-1-ol. Aldehydes: propanal, butanal, pentanal, hexanal, heptanal, octanal, nonanal, decanal. Amines: propylamine, butylamine, pentylamine, hexylamine. Carboxylic acids: ethanoic acid, propanoic acid, butanoic acid, pentanoic acid, hexanoic acid, heptanoic acid, octanoic acid, nonanoic acid, decanoic acid, isobutyric acid, isovaleric acid, 5-hexanoic acid, (*E*)-3-hexenoic acid, 2-oxovaleric acid, DL-lactic acid. Esters: ethylacetate, methylpropionate, methylbutyrate, ethylpropionate, ethylhexanoate, ethyl-3-hydroxybutanoate, ethyl-3-hydroxyhexanoate, ethyllactate, methylsalicylate, (*E*)-2-hexenylacetate, ethylbutyrate. Floral volatiles: guaiacol, veratrole, phenylacetaldehyde, 1-phenylethanol, 2-phenylethanol, benzylalcohol, isobutyric acid phenylester, propionic acid phenylester, phenylether. Green leaf volatiles: hexanol, hexanal, (*E*)-2-hexen-1-ol, (*Z*)-2-hexen-1-ol, (*E*)-2-hexenal, (*E*)-2 –hexenylacetate, (*Z*)-3-hexenyl acetate, 1-hexene-3-ol. Indoles: indole, 1-, 2-, 3-, 4-, 5-, 6- and 7- methyl indole. Ketone: 2,3-butanedione, α+β-thujone, geranylacetone, 6-methyl-5-heptene-2-one, 5-methyl-2-hexanone, 2-heptanone. Lactone: γ-valerolactone, γ-hexalactone, γ-octalactone, γ-decalactone. Phenols: phenol, *o*-, *m*- and *p*-cresol, 4-ethylphenol. Sulfer: dimethyltrisulfide, dimethyldisulfide, carbondisulfide. Terpene: germacerene D, α-pinene, β-pinene, β-elemene, β-caryophyllene, α-caryophyllene, α-phyllandrene, γ-terpinene, *p*-cymene, farnesene, β-myrcene. Most of the chemicals were purchased from Sigma–Aldrich and were all >95% pure. Racemic 1-octen-3-ol and linalool (50:50 as indicated by chiral GC) were from Fluka (98% pure). DL-lactic acid was 90% Pure (Fluka); 3- and 4-Methyl indole were purchased from Acros and were 98% pure. The α+β-Thujone was technical grade (Fluka). Ammonia (25%) and trimethylamine [(TMA), 45%] were aqueous solution purchased from Fluka. Optically pure (*R*)-(—)-1-

octen-3-ol, (*S*)-(+)-1-octen-3-ol, D-(+)-linalool and L-(−)-linalool were kindly provided by Bedoukian Research. Chemicals were dissolved in dichloromethane (DCM), wt/vol, to make a stock solution of 10 μg/μL and decadic dilutions were made. Ammonia, TMA, and alkyl amines were dissolved in water. An aliquot (10 μL) of a stimulus was loaded onto a filter paper strip, the solvent was evaporated for 30 s, and the strip was placed in a 5 mL polypropylene syringe from which various volumes were dispensed. Solvent alone and an empty syringe served as control. Throughout this article, dose of the compounds refer to the amounts loaded onto stimulus cartridges. Stimuli were added for 500 ms at 2 mL/s on a continuous humidified airflow at 20 mL/s blowing over mosquito preparation resulting in ≈10× dilution of the stimulus concentration reaching the preparation.

1. Syed Z, Leal WS (2008) Mosquitoes smell and avoid the insect repellent DEET. *Proc Natl Acad Sci USA* 105:13598–13603.

2. Syed Z, Leal WS (2007) Maxillary palps are broad spectrum odorant detectors in *Culex quinquefasciatus*. *Chem Senses* 32:727–738.

Fig. S1. Set-up for SPME collection of volatiles from the forearm of a human volunteer. A SPME gray fiber (*Inset*) is held by a 1 mL pipette tip and cloth tape to avoid direct contact with the skin. Forearm is enclosed in an aluminum foil with both ends loosely tied with cloth tape.

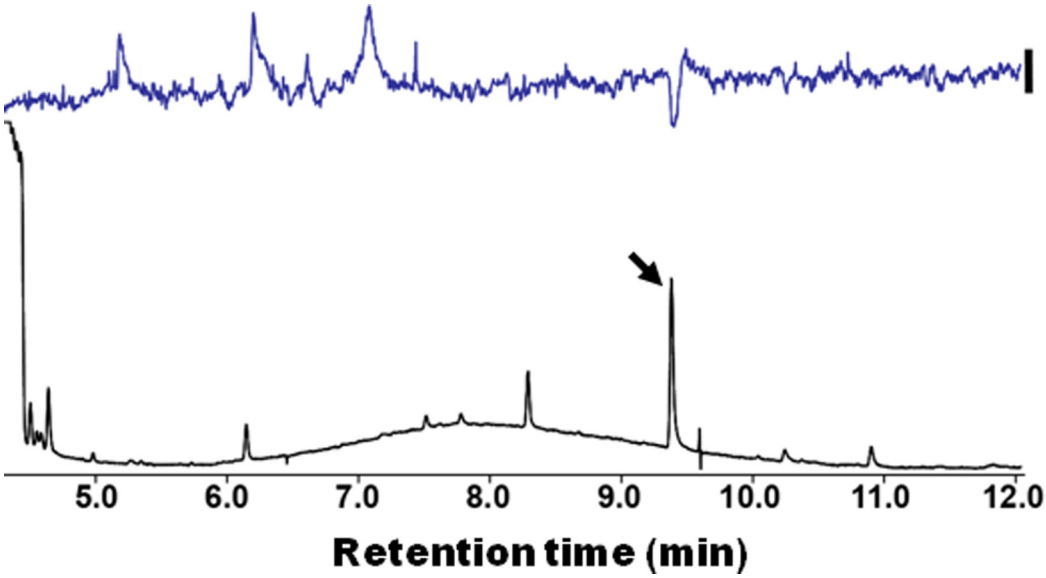

Fig. S2. Analysis of pigeon odorants collected by SuperQ, separated by gas chromatography, and simultaneously detected by FID and EAD (blue trace). Upward deflections are due to mechanical disturbances during the GC-EAD run. Nonanal (arrow) was the only EAD-active peak ($n = 5$). (Scale bar, 0.2 mV.)

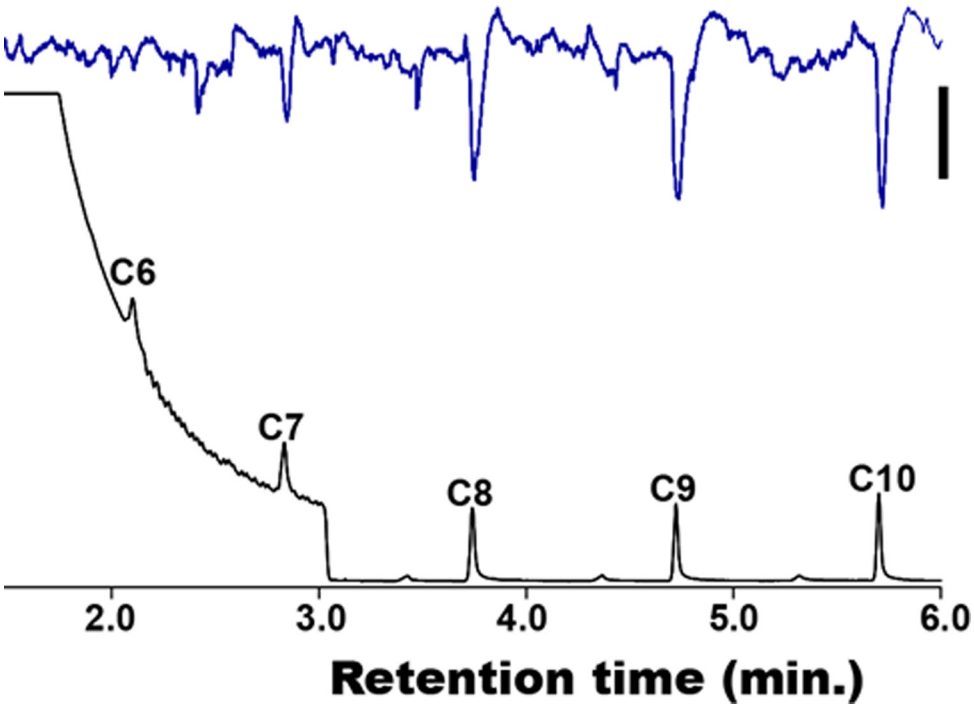

Fig. S3. GC-EAD responses of *Cx. quinquefasciatus* antenna to homologous series of aliphatic aldehydes at 50 ng each. (Scale bar, 0.2 mV.)

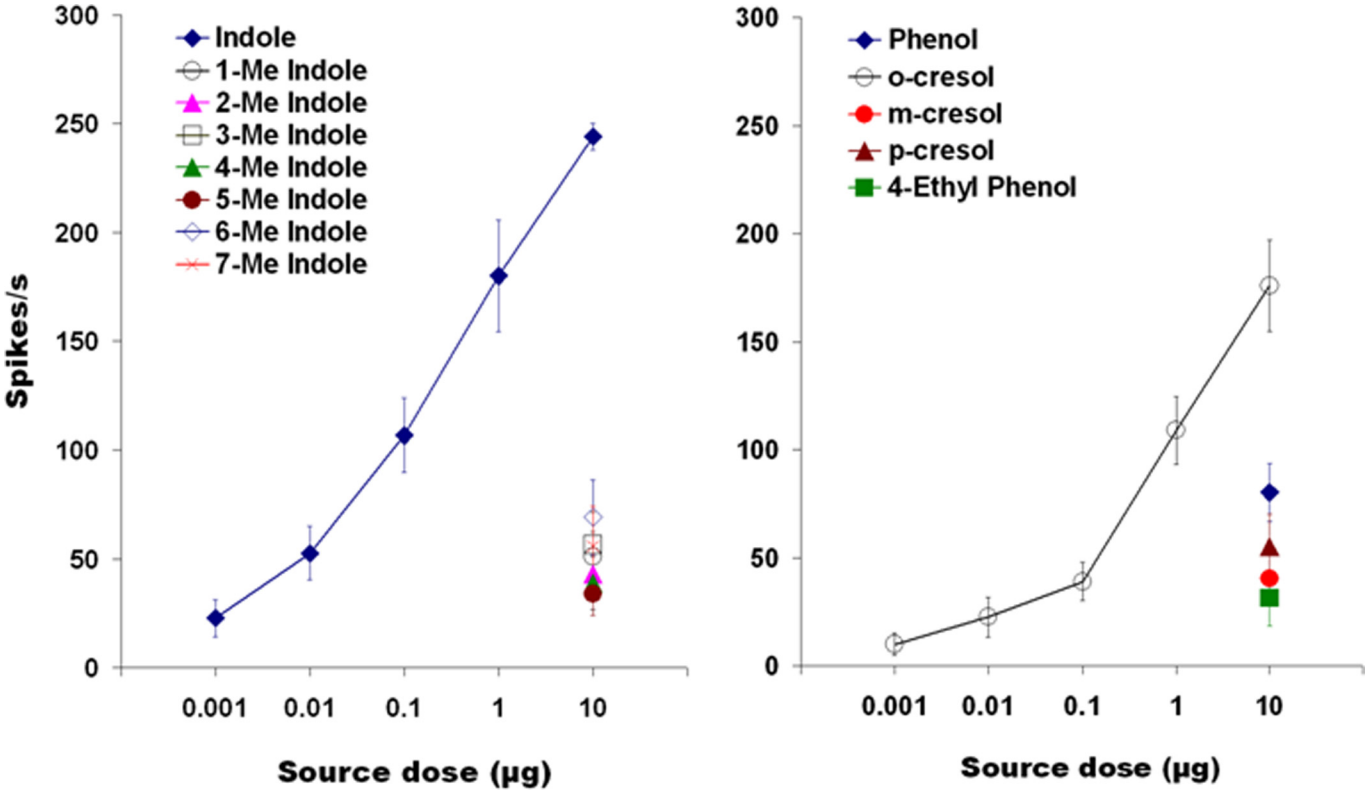

Fig. S4. Single sensillum recordings showing dose-dependent responses of ORNs colocated in nonanal-sensitive A2 sensilla responding to indole and *o*-cresol ($n = 6 \pm$ SEM). Sensitivity to indole dramatically decreased with the addition of a methyl group at any position, whereas the hydroxyl group in cresols showed the highest response at the ortho position.

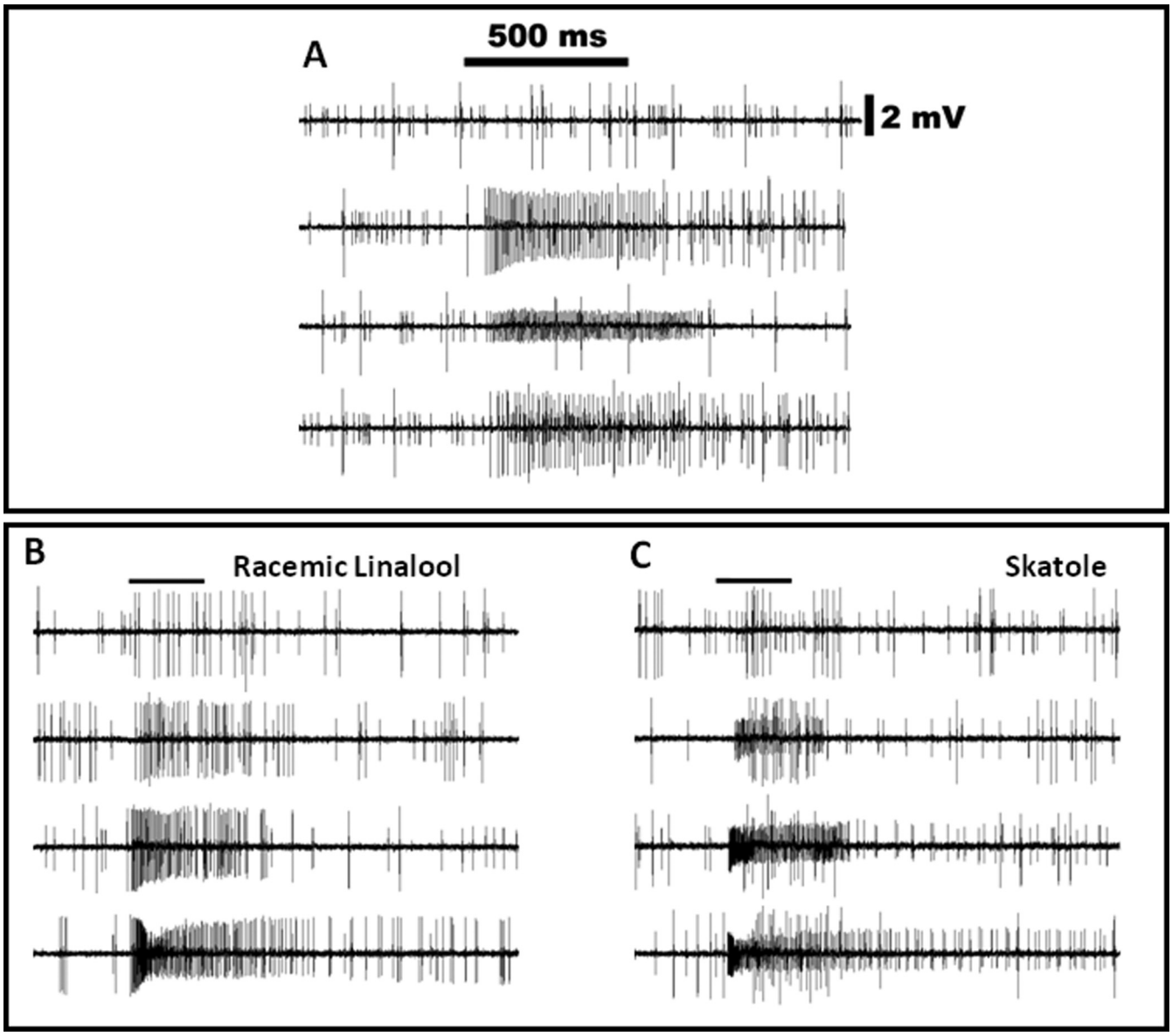

Fig. S5. A1 type trichoid sensilla detected various plant compounds with colocated ORNs. (*A*) From top to bottom: Spontaneous activity trace displaying the ORNs characterized by distinct spike amplitudes and frequencies; excitatory response of the ORN with larger amplitude to 1 μg source dose of racemic linalool; colocalized ORN responding to 1 μg skatole; and the trace at the bottom shows both ORNs responding to 100 ng of racemic 1-octen-3-ol. (*B* and *C*) Excitatory dose-dependent responses to racemic linalool and skatole to the increasing doses (top to bottom: 0.01, 0.1, 1, and 10 μg).

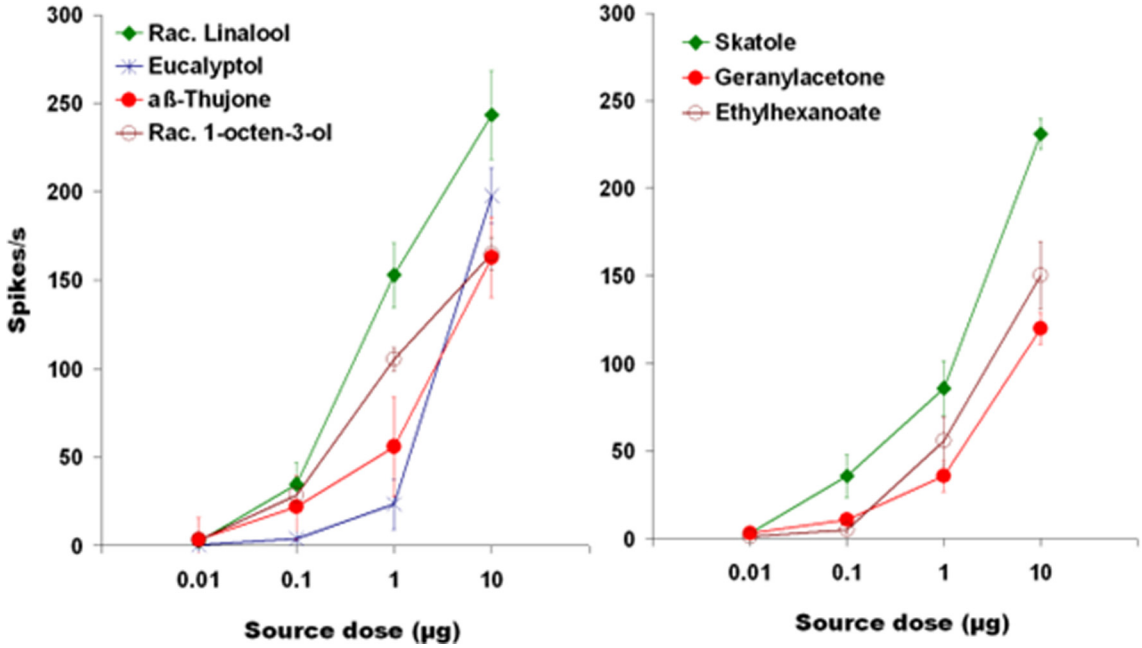

Fig. S6. Dose-response curves for the strong ligands identified for A1 sensillum type ($n = 5 \pm$ SEM). For further details, see Table 1 and Fig. S5.

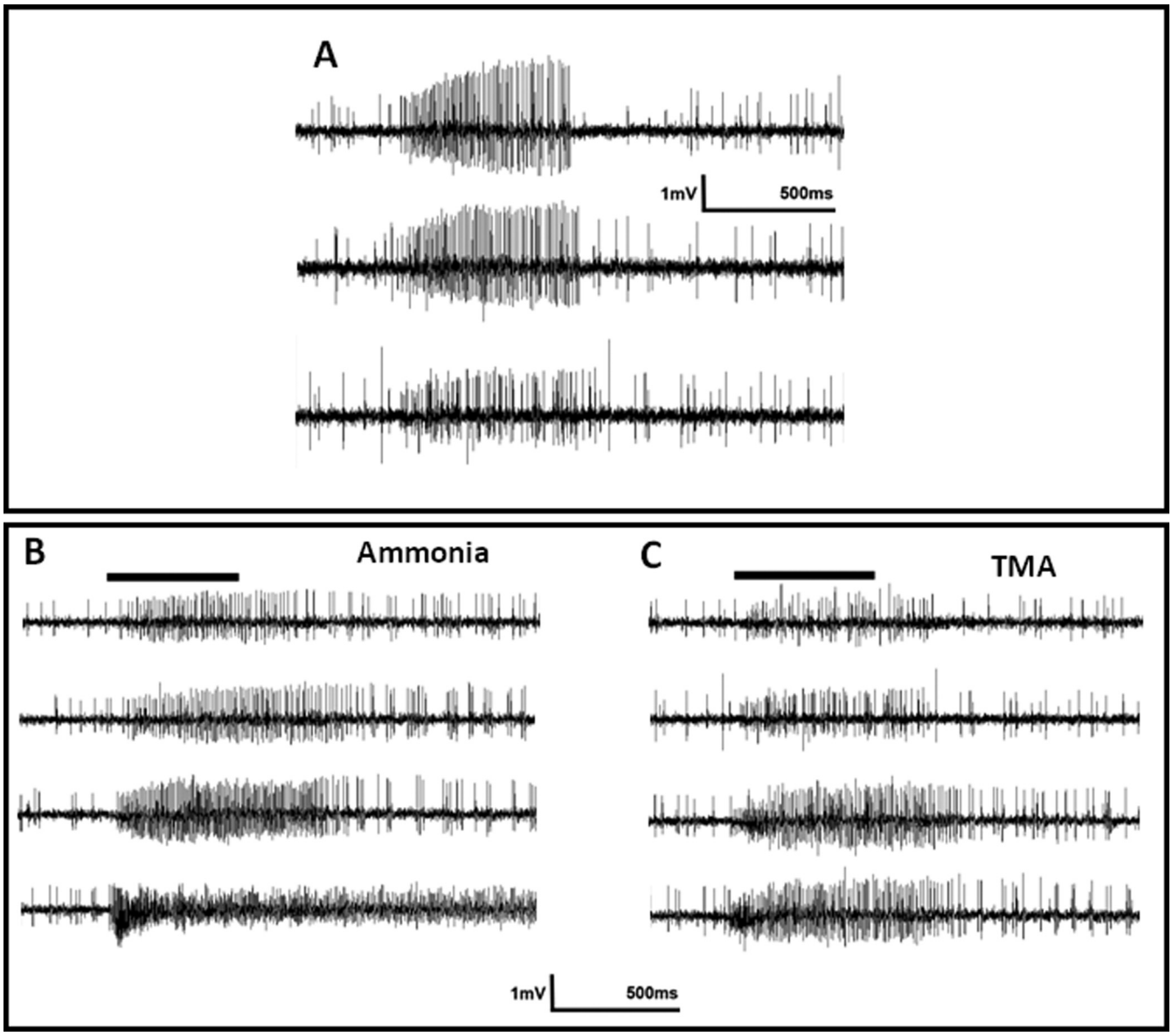

Fig. S7. Grooved peg sensilla, A3, detect polar compounds with 3 ORNs. (*A*) From top to bottom: ORN with largest spike amplitude responded to 0.025% source dose of ammonia; intermediate amplitude ORN responding to 1 μg source dose propylamine; and in the bottom trace, smallest amplitude ORN responding to 0.045% TMA. (*B* and *C*) Excitatory dose-dependent responses of two colocated ORNs to ammonia (from top to bottom, 0.0025, 0.025, 0.25, and 2.5%) and TMA (from top to bottom, 0.0045, 0.045, 0.45 and 4.5%).

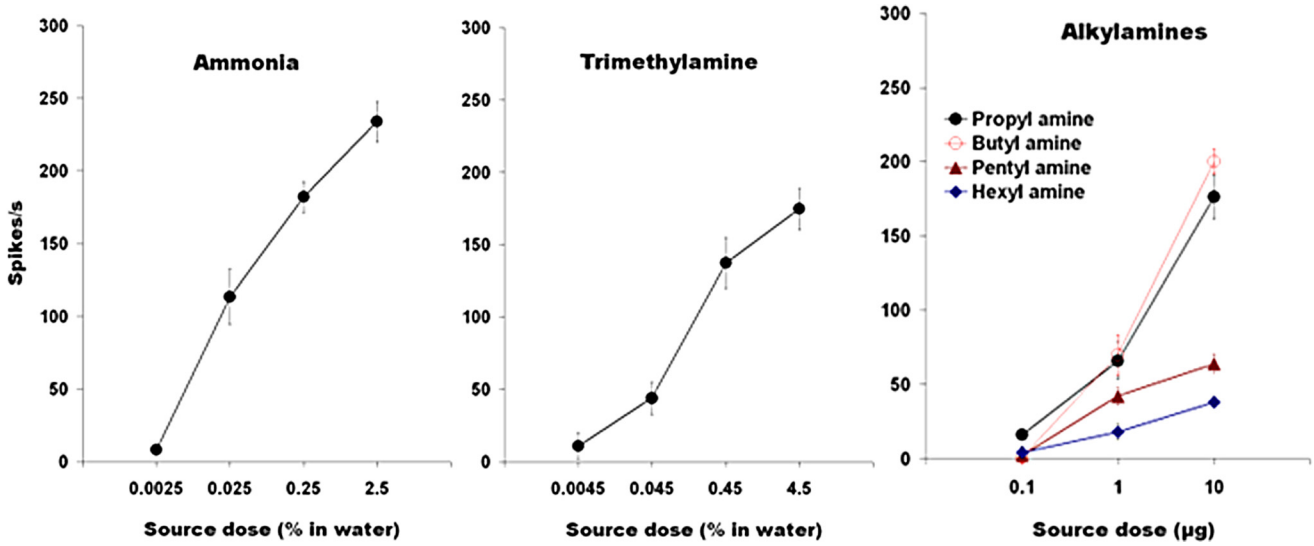

Fig. S8. Dose-response curves for the strong ligands identified for A3 grooved peg sensillum type ($n = 8 \pm$ SEM). For further details, see Table 1 and Fig. S7.