Supporting Information

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SI Materials and Methods

Insects. Insects were reared for several generations in the laboratory and the culture was supplemented yearly with wild collected insects. Larvae were reared on a potato-based diet (1). Pupae were separated according to sex and kept in separate rearing chambers at 23 °C, 70% relative humidity, and a 16-h/8-h light/dark cycle.

Walking Bioassay. In the open arena olfactometer (2), air was pushed through a baffle with holes spaced at 2 mm while an exhaust in the other end sucked out contaminated air, resulting in a laminar flow of 0.5 m/s. Experiments were done 11 h/2 to 4 h into the scotophase in red light (3 l×) at 18 to 20 °C. During the photophase, males were placed individually in glass tubes with one end covered with a mesh net and transported to the experimental room before the start of the scotophase. The moths were allowed to acclimatize in the testing room for approximately 2 h before the onset of the experiments and experienced the onset of the scotophase in the experimental room.

Odor Stimuli. In *S. littoralis*, the pheromone blend composition shows geographical variation (3, 4). The main pheromone compound is always *ZE*-9,11–14:OAc, and in addition, a number of minor compounds have been identified, but the minor components differ in presence and ratio among different populations. In the population of insects we used, the complete sex pheromone blend composition has not yet been fully identified. For this reason, we used female sex pheromone gland extracts for our behavioral experiments. For each extract, 20 to 30 glands from 2-d-old virgin females were dissected and extracted in 100 μL hexane 2 to 3 h into the scotophase. For storage, the extract was transferred to a small glass vial and diluted to 1 fe per 10 μL. GC analysis showed that 1 fe corresponded to approximately 20 ng of *ZE*-9,11–14:OAc. For electrophysiological experiments, dilutions in decadic steps from 0.01 pg to 1 μg of *ZE*-9,11–14:OAc (>97% purity checked by GC; CAS 50767–79-8 was synthesized in the laboratory in Versailles; gift from Martine Lettere) in hexane and from 0.1 to 1,000 μg of linalool (racemic, 97% purity; Sigma-Aldrich), geraniol (96% purity; Sigma-Aldrich), and heptanal (95% purity; Sigma-Aldrich) in mineral oil (95% purity; Sigma-Aldrich) were used. The three plant-related compounds have previously been shown to be emitted by flowers and to be detected by *S. littoralis* and other noctuid moth antennal ORNs (5–8).

Sound Stimuli. Sound stimuli were generated by multiplying square wave signals from a pulse generator (model 555; Berkeley Nucleonics) with sine wave signals from a function generator (model 33120A; Agilent) in a custom-built trapeze modulator. The signal was attenuated (865 step attenuator; Kay), amplified (S55; UltraSoundAdvice), and broadcasted through an electrostatic loudspeaker (S56; UltraSoundAdvice). The loudspeaker was placed close to the surface of the olfactometer floor. The sound intensity was measured by a 0.25-inch microphone (G.R.A.S.) that was calibrated against a G.R.A.S. sound calibrator. The sound pressure was measured at several points in the middle of the arena in front of the loudspeaker at the surface level, and according to these results, an area was marked in which the sound pressure did not vary by more than ±3 dB around the mean of 102 dB SPL.

Pretreatments. In the experiments, males were submitted to different pretreatments before the trial. During all pretreatments, the moths were kept inside glass tubes that were covered by a mesh on both sides and were individually placed in the center of the olfactometer. We have shown earlier that upwind movement toward the pheromone source is not necessary for obtaining preexposure effects (2). During preexposure to the pheromone, 1 fe of gland extract was placed at the upwind end of the olfactometer and the glass tubes with one male each were individually put 40 cm downwind in the pheromone plume. During the exposure time of 10 s, a large majority of the males showed activation and upwind movement within the glass tube. For sound exposure, moths were placed in the marked area on the arena where sound pressure did not vary more than ±3 dB. After a short time allowing the moths to settle, the sound was turned on manually for 10 s.

The following stimuli were used for preexposure:

i) Sex pheromone gland extract (1 fe).
ii) Pulsed bat-like sound consisting of pulse trains consisting of 20 trapezoid-shaped pulses. Each pulse was 4.7 ms long with a carrier frequency of 30 kHz. The pulse trains were repeated 10 times, resulting in a total exposure time of 940 ms. Sound intensity was 102 dB sound pressure level. This stimulus elicited a consistent behavioral response: 30 kHz was chosen because it is within the moths' best frequency of hearing (9) and because many bats, including gleaners, emit echolocation signals including 30 kHz. The temporal structure of our stimuli corresponds roughly to the signals emitted during the search phase of many bats (10–12). Furthermore, these stimulus parameters evoke maximum silencing response in the acoustically signaling moth, *Achroia grisella* (13).
iii) A tone consisting of a continuous 940-ms, 30-kHz signal at 102 dB sound pressure level.
iv) Simultaneous stimulation to sex pheromone gland extract (1 fe) and pulsed bat-like sound as described earlier.

After the pretreatments both treated and control males were kept individually in the glass tubes and transported back to the rearing chamber. The next day, just before the onset of the scotophase, they were returned to the experimental room for test trials. Each treatment was tested on several occasions to minimize the effects of variations in conditions between days and variation between different batches of moths. Males were tested 24 to 27 h after preexposure, and normally, pheromone exposure and pheromone tests were done on different days. In the rare cases in which preexposure with pheromone was done the same day as subsequent behavioral tests of males from the previous day, the airflow of the olfactometer was allowed to run at least for 1 h to clear any remaining pheromone traces.

Behavioral Tests. The behavioral tests were performed in the same arena used for the preexposure. In an earlier study, we have shown that male moth behavior in the walking assay and in a wind tunnel were similar, even if response thresholds differed (2). Pheromone solutions at an amount of 0.03 fe were applied on pieces of filter paper, which were placed upwind at the center of the arena in front of the baffle. This dose was chosen because it resulted in a low percentage of responses of the naive males and allowed us to observe a clear effect of preexposure. The filter paper was changed every 20 min. Males were introduced individually in the arena at the center of the odor plume 40 cm downwind from the odor source. Moths were given 120 s to respond and the males that reached within 5 cm of the odor source or made source contact were scored.

Electrophysiology. Intracellular recordings of neurons situated in the MGC and in OG of the AL were performed according to standard methods (14). The stimulation procedure and experimental protocol of intracellular recordings have been described in detail earlier (15). Briefly, thresholds of AL neurons were determined by stimulating the ipsilateral antenna with increasing doses of sex pheromone or plant compounds with at least 10 s between individual stimulations or until spontaneous activity recovered. For stimulation, an airflow (7 mL/s) carrying the odor stimulus was inserted for 500 ms into a continuous airflow (17 mL/s) by using a stimulus controller (CS 55; Syntech). Recordings were stored and analyzed on a PC with Autospike 32 software (Syntech). For the statistical analysis of neuron thresholds, electrophysiological responses were quantified as described previously (16). Briefly, spontaneous activity during the 500 ms preceding odor stimulation was subtracted from the response during 500 ms during stimulation to determine a response. As most AL neurons responded also to the solvent hexane (or to the mechanical stimulus during the switch of airflows), a neuron was classified as responding to a stimulus when the odor response exceeded the response to a hexane stimulus by at least 10%. Data are presented as cumulative threshold curves as a function of stimulus dose threshold distributions.

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