

Fig. S1. Proboscis extension response (PER) rates during olfactory conditioning trials (O+ and C-, N=36; C+ and O-, N=54). CS+: reward associated conditioned stimulus, CS- reward non associated conditioned stimulus.

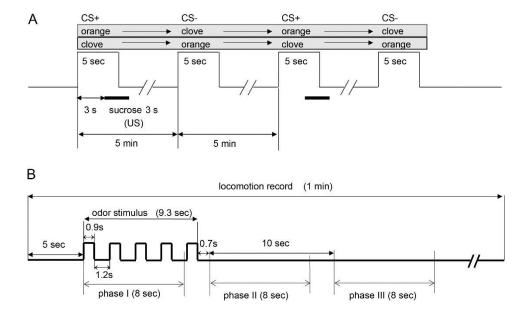


Fig. S2. A: Odor conditioning of PER. For olfactory conditioning, either orange or clove odors (CS⁺) was applied to the antennae of honeybees for 5 s. Three seconds after the onset of CS⁺, 1.5 M sucrose solution was applied for 3 s (the unconditioned stimulus, US). After 5 min, the alternative odor was applied without sucrose solution (CS⁻). These two series of differential conditioning, shown as the upper and lower bars, were applied at 5 min intervals for up to five repeats of the cycle. It was evaluated whether the PER was induced during 3 s after the onset of odor stimuli. B:

The time schedule of locomotion recording. Five odor pulses were applied over about 10 s, commencing 5 s after the onset of recording. Locomotion was recorded for 1 min. The time-course of the locomotion were compared during three 8-s phases. phase I, during the odor stimuli; phase II, commencing at the end of the odor stimuli; and phase III, 8 s commencing 10 s after the end of the odor stimuli.

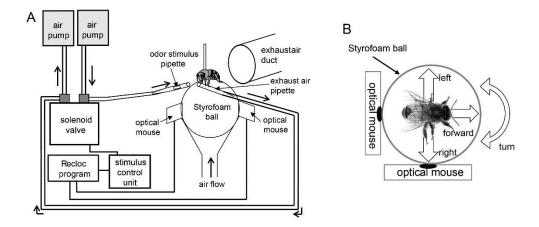


Fig. S3. Locomotion recording setup. A: A honeybee was placed on top of a Styrofoam ball suspended by air flow. The position was adjusted to enable the honeybee to walk naturally. After the honeybee ceased walking on the ball, the odor was applied with intermittent pulses from the pipette in front of honeybee. To prevent interference from odor leaking from the pipette, a second exhaust pipette was situated in front of the honeybee. Both stimulus timing and locomotion recording were controlled by a locomotion recording program (Recloc). B: Two optical mice were placed close to the surface of the ball laterally and posteriorly to the honeybee. The rotation of the ball caused by the honeybee's locomotion was used for quantitative analysis by Recloc.

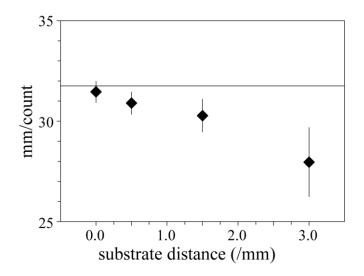


Fig. S4. Mouse sensor movement detection. Displacement information obtained from optical mice (Logitech G5) moved over a Styrofoam substrate at different distances from the substrate. Data represent means and standard deviations obtained from 20 sessions per point. Nominal resolution was 31.75 µm/count (800cpi) as indicated by a horizontal line. Displacement was controlled via 0.1mm/turn leadscrews of a micromanipulator (MX-1, Narishige, Tokyo, Japan) with the x/y-axes aligned as well possible to the mouse sensor axes and compensating for backlash before every trial. At zero substrate distance, readings varied from 30.30 to 32.26µm (standard deviation 0.54µm, n=20), resulting in approximately 5% maximum error with respect to the nominal value. While average reported displacement declined with increasing substrate distance, the variance around the mean reported value only started to increase at substrate distances over 1mm. Systematic tests on the dynamic performance of displacement detection by the mouse sensors could not be carried out. Readout was 100% reliable with submicrosecond jitter at 500Hz under the experimental conditions as confirmed by saving time stamps obtained from the PC's real-time clock immediately after obtaining valid mouse data using optional real-time functionality of the Linux kernel (2.6.18 or 2.6.29).

Table S1. Abbreviations of odor used as stimulant.

			Learner	
Odor group	Control	Non-learner	Orange CS+	Clove CS+
			Clove CS—	Orange CS—
Orange	O (n=28)	O ⁰ (n=19)	O ⁺ (n=16)	O ⁻ (n=19)
Clove	C (n=24)	C ⁰ (n=19)	C ⁻ (n=16)	C ⁺ (n=19)



Movie 1. Typical example of locomotion induced by O+. When a stimulus pulse was applied, the LED was turned off. Locomotion was always recorded in a dark room. The compound eyes were also masked by paint.