Supplementary material

Supplementary Figure 1

Figure S1: A) Bee tent used for odor exposure. Cirled in green, orange and blue are, respectively: the sucrose solution-containing Boardman bottle, the odor stimulus, water. B) Daily schedule of odor/food/light exposure. Bees were initially provided with food and water. The odor dispenser was added after one hour. Each following day, food and odors were removed independently for certain periods, according to the schedule, and renewed afterwards. After 72h, bees were either sacrificed for immunostaining, or injected with fura-2 for calcium imaging. After injection, bees were placed back into the tents overnight for dye diffusion. They were finally removed from tents in the next morning, a few hours before the imaging experiments.
Figure S2: Paradigm of odor stimulation delivered to the bee antennae through a stimulus generator. Each stimulus had duration 1 s. The initial delay was 2 s for the novel odor and 8 s for pre-exposed odor. By using stimulus periods of 4 s for the novel odor and 2 s for the pre-exposed odor, a stimulus pattern is created which presents the novel odor, the pre-exposed odor and the binary mixture in a pseudo-random manner. CTR bees underwent the exposure protocol of either HEX or NNL pre-exposed bees by random choice.
Figure S3: Odor evoked response [ΔF/F] of glomerulus T1-33 recorded under different stimulation paradigms. Four glomeruli T1-33 of four example bees are presented (one NNL pre-exposed bee, one HEX pre-exposed bee, and two CTR bees, which were subjected to either the stimulation paradigm of NNL pre-exposed bees or to that of HEX pre-exposed bees). The response to the three odors shows a pattern that is typical of the treatment group and not of the “order” group. In particular, in CTR and HEX pre-exposed bees, the profile response to 1-nonanol (NNL) is found almost unchanged in the response to the binary mixture (MIX). In NNL pre-exposed bees, response to binary mixture is flat, as it is the one to 1-hexanol. The plots represent three odors repetitions averaged ± SEM (shadow). Red vertical bars represent odors onset and offset.
Glomerulus T1-17 odour-evoked responses.

Figure S4: Odor evoked response [-ΔF/F] of glomerulus T1-17 recorded under different stimulation paradigms. Four glomeruli T1-17 of four example bees are presented (one NNL pre-exposed bee, one HEX pre-exposed bee, and two CTR bees, which were subjected to either the stimulation paradigm of NNL pre-exposed bees or to that of HEX pre-exposed bees). The response to the three odors shows a pattern that is typical of the treatment group and not of the “order” group. In particular, in CTR and HEX pre-exposed bees, the profile response to 1-nonanol (NNL) is found almost unchanged in the response to the binary mixture (MIX). In NNL pre-exposed bees, response to binary mixture is flat, as it is the one to 1-hexanol. The plots represent three odors repetitions averaged ± SEM (shadow). Red vertical bars represent odors onset and offset.