Online Methods

Subjects

All experiments were conducted in accordance with the guidelines of the National Institute of Health and were approved by the Institutional Animal Care and Use Committees at both the Nathan Kline Institute and the New York University Medical School. A total of 58 Male Long-Evans hooded rats (206–528 g at recording) obtained from Charles River Laboratories were used as subjects. Animals were singly housed in polypropylene cages and maintained on a 12 h light/dark schedule. They were given *ad libitum* access to food and water except during the behavioral procedure when access to water was given twice a day (30 min in the experimental chamber and 30 min in the home cage). Subjects were handled (5 min/d) and weighed daily to assess their adaptation to water restriction. Behavioral training and electrophysiological recordings were made during the light phase of the 12 h light/dark cycle.

Behavioral procedure

The behavioral procedure has been detailed previously[3]. Odor discrimination ability was assessed with a two alternative forced-choice odor discrimination task for water reward. Animals were trained in sessions of 30 min, 5 days/week. The behavioral apparatus consisted of a plastic box (30x30x40cm; Vulintus, Sachse, TX [http://Vulintus.com]) containing a central odor port on one wall and two opposite water ports on the left and on the right walls (**Fig. 1c**). Rats initiated a trial by poking their nose in the odor sampling port to initiate odor onset; the animal must hold in the odor port for at least 350 ms for trial initiation. Depending on odor identity, the rat then had to make a choice of a left or right reward port within 3 s to initiate water delivery. Odor presentations were randomized and at least 25 trials with each stimulus were included in each test. Rats usually performed about 100 trials within the 30 min sessions. Mean error rate within a session was used as the measure of discrimination and compared across sessions with ANOVA for repeated measures and Student's *t* test for paired samples.

Pre-training

During the pre-training phase, the animals learned to perform the task with an easily discriminable pair of monomolecular odorants (vanilla versus peppermint) until criterion performance (error rate<0.25 for both sides) was attained during three successive sessions. In average, animals were able to reach this criterion after (mean ± s.d.) 15 ± 3 days of training; the animals showing this performance belonged to the conditioned group (n= 39 rats). Some animals (n= 8 rats) failed to acquire this same task after 25 days of training (error rate never below <0.4) and were allocated to the pseudo-conditioned group.

Mixtures discrimination

Mixtures were created by adding odorant components to mineral oil in amounts that provided identical component concentrations (approximately 100 p.p.m.) within the mixture based on individual odorant vapour pressure[3]. The initial 10 component mixture (10c) included the following monomolecular odorants (vapour pressure indicated between parenthesis): isoamyl acetate (5.00 mm Hg), nonane (4.29), ethyl valerate (4.80), 5-methyl-2-hexanone (4.60), isopropylbenzene (4.58), 1-pentanol (6.11), 1,7-octadiene (6.15), 2-heptanone (3.86), heptanal (3.52) and 4-methyl-3-penten-2-one (6.69).The 10c mixture was then degraded by the removal of one (isoamyl acetate) or two (isoamyl acetate and nonane) components(10c-1, 10c-2) or transformed by the replacement of one component (isoamyl acetate) by another component (limonene [1.98]) (10cR1). During the test phase, the rat's ability to discriminate the 10c core mixture from its subsets was evaluated throughout a short (two days) or a longer training (eight days).

Recording and odorant stimulation

The electrophysiological recordings in OB and the aPCX were performed in different animals. There were no differences in learned behavior between animals randomly chosen for OB recordings or for aPCX recordings. Details of single-unit recording and odorant-response characterization techniques for layer II/III anterior piriform cortex neurons and for mitral/tufted cells have been reported elsewhere [10,19]. Two categories of animals were recorded: one was never trained nor exposed to odors (naïve group, n=9 rats) while the other was trained in the odor discriminative task

described above [conditioned (n=32 rats) and pseudo-conditioned (n=8 rats) groups]. Electrophysiological recordings were always performed the day following the end of the training. All the animals had had access to water during the 20 h preceding recording. Animals were anesthetized with urethane (1.5 g/kg) and were freely breathing with the respiratory cycle monitored through a piezoelectric sensor strapped to the chest. Single units (filtered at 300 Hz to 3 kHz) and local field potentials (LFPs, filtered at 0.1–300 Hz) were recorded simultaneously with a single tungsten microelectrode (1–5MΩ). Signals were digitized at 10 kHz with a CED micro1401 and analyzed with Spike2. Mitral/tufted cells were identified by antidromic stimulation of the lateral olfactory tract. Layer II/III anterior piriform cortex neurons were identified by lateral olfactory tract-evoked responses and histological confirmation.

Odorants were delivered with a flow-dilution olfactometer, with a constant, 1 litre per minute (LPM) flow of filtered air presented 1–2 cm from the animal's nose. Saturated odorant vapor was added at 0.1 LPM to the clean air stream via computer controlled solenoids to produce an approximate dilution of 1:10 of saturated vapor. Odorant stimulus onset was triggered off the respiratory cycle to coincide with the transition from inhalation to exhalation. The stimulus duration was 2 s with at least 30 s interstimulus intervals to reduce cortical adaptation. All the odor stimulations were presented during the fast-wave states of anesthesia since the single units showed reduced responsiveness to odors during the slow-waves periods[47]. Each unit was tested with each stimulus presented 3-5 times randomly. The stimuli used during recordings were the same odorants with the same concentration as those used for the behavioral task.

Data analysis

Electrophysiological data were analyzed as previously described[10,48]. Neural responses to odors were assessed at the single-unit level, at the ensemble of neurons level and at the whole neural network level through the examination of odor-evoked oscillatory activities. Single-unit spike sorting, cluster cutting, waveform analysis and Fast Fourier Transform power analyses of the local field potentials were all performed in Spike2 (CED, Inc.).

The single-unit nature of the recordings was verified by at least a 2 ms refractory period in interval histograms. Single-unit responses to odors were analyzed with peri-stimulus histograms: the response magnitude of mitral cells and aPCX neurons to odorants were quantified as the difference in number of spikes evoked during the 0-3 s post-stimulus onset compared with a 3 s prestimulus period. Odor-responsive neurons were defined by firing rates with a 30% odor-evoked increase above spontaneous activity. Suppression indicates a 30% or more decrease in odor-evoked activity to the baseline.

The breadth of tuning metric of Smith & Travers[49] was calculated to assess the proportional distribution of a cell's response across the four complex stimuli used in this study (10c, 10c-1, 10c-2 and 10cR1). The entropy coefficient was calculated as follows:

$$H= -K \sum_i p_i \log p_i$$

where H is the breadth of tuning, K is the scaling constant (K=1.661 for four stimuli, set so that H=1.0 when the neuron responds equally well to all stimuli) and p_i is the proportion of the response to stimulus *i* relative to the summed response to all four stimuli. The entropy measure varies from 0.0 for a cell responding exclusively to one stimuli (*i.e.*, narrowly tuned) to 1.0 for a cell responding equally to all of the four stimuli (*i.e.*, broadly tuned). To examine differences in breadth of tuning between naïve and trained animals, Student's *t* tests for unpaired samples were performed on the entropy values.

Virtual ensemble data[10] were created from combined single-unit recordings across animals Pearson correlation coefficients (*r*) were calculated for responses across stimulus pairs for aPCX neurons and mitral cells to examine if the populations of neurons encoded the10c initial mixture similarly (significant correlation, assessed using the Fisher's *r* to Z *test*) or differently (non-significant correlation) from its morphed versions (10c-1, 10c-2, 10cR1). The stability of odor responses was verified through the significant correlation between multiple repetitions of 10c. Statistical comparisons between the 10c self correlation and the correlations obtained with its morphed versions were then assessed with the test of the difference between correlation coefficients[50].

Odor-evoked oscillatory activities were estimated in the beta (15–35 Hz) and gamma (40-80 Hz) frequency bands. For each olfactory stimulation, power spectra (FFT size: 0.2048s; Hanning window) were calculated for the 0-3s post-stimulus onset period and normalized by the power of the 3 s prestimulus baseline for comparisons between animals and experimental conditions. As the distribution of power value is not normal, statistical comparisons between naïve and trained animals were performed using the Mann–Whitney *U* test for unmatched samples.

Histology

After recording, animals were overdosed with anesthetic, transcardially perfused with saline and 4% paraformaldehyde. Coronal brain sections (40 μm thick) were performed and stained with cresyl violet for determination of electrode positions (**Supplementary Fig. 1a**).

References

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