

Information for decision-making and stimulus identification is multiplexed in sensory cortex

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In recordings from anterior piriform cortex in awake behaving mice, we found that neuronal firing early in the olfactory pathway simultaneously conveyed fundamentally different information: odor value (is the odor rewarded?) and identity (what is the smell?). Thus, this sensory system performs early multiplexing of information reflecting stimulus-specific characteristics with that used for decision-making.

The olfactory bulb converts a complex input from ~1,400 olfactory receptors¹ into odor value after one or two synapses^{2,3}. Synchronous firing of mitral cells then transfers information to the cortex³⁻⁵. The early olfactory system thus faces the challenge of transmitting information about both stimulus value and identity. Multiplexing is one mechanism for simultaneously transmitting this information⁶, but how information on odor value and identity is multiplexed is not understood.

In humans, anterior piriform cortex (APC) responds to olfactory stimuli when actively detecting odors, but shows reduced functional magnetic resonance imaging (fMRI) signals when the subject is passive⁷. Yet, even in passive sampling, odor identity is conveyed to detect deleterious odors. We asked whether transmission of information

regarding identity and value through APC is multiplexed and whether coding differs during active and passive monitoring.

In the active odor detection task, the mouse received water for licking when presented with rewarded odors, but not when it was exposed to an unrewarded odor (Fig. 1 and Supplementary Figs. 1-4). The mice responded correctly in $87 \pm 6\%$ of trials (\pm s.e.m., $n = 20$ sessions). As expected^{4,5}, the elicited changes in firing rate differed between rewarded and unrewarded odors (Fig. 1). A substantial number of units responded to odor with altered firing rate (22% of 139 single and 63% of 216 multi-units). Consistent with previous findings in mitral cells³, this result indicates that firing rate conveys information on odor value. To determine when changes in firing rate of APC cells convey sufficient information, we determined the Euclidean distance between rewarded and unrewarded odors in multidimensional space with odor-elicited changes in firing rate for APC cells in each dimension. Euclidean distance increased substantially before the behavioral decision time (Fig. 1c).

Odors elicit substantially reduced fMRI signals in passive tasks in humans⁷, raising the question of whether odor-induced changes in the firing rates of APC neurons decrease during passive tasks. We tested APC neuron responses during a passive task in which mice did not actively discriminate between odors (rewarded for licking for any odor). Consistent with human studies⁷, we observed substantially reduced responses to rewarded odors in the passive task (responses

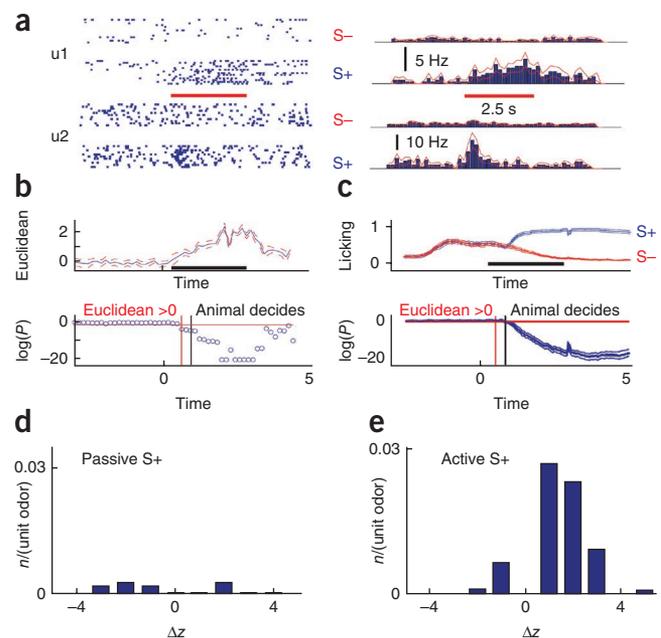
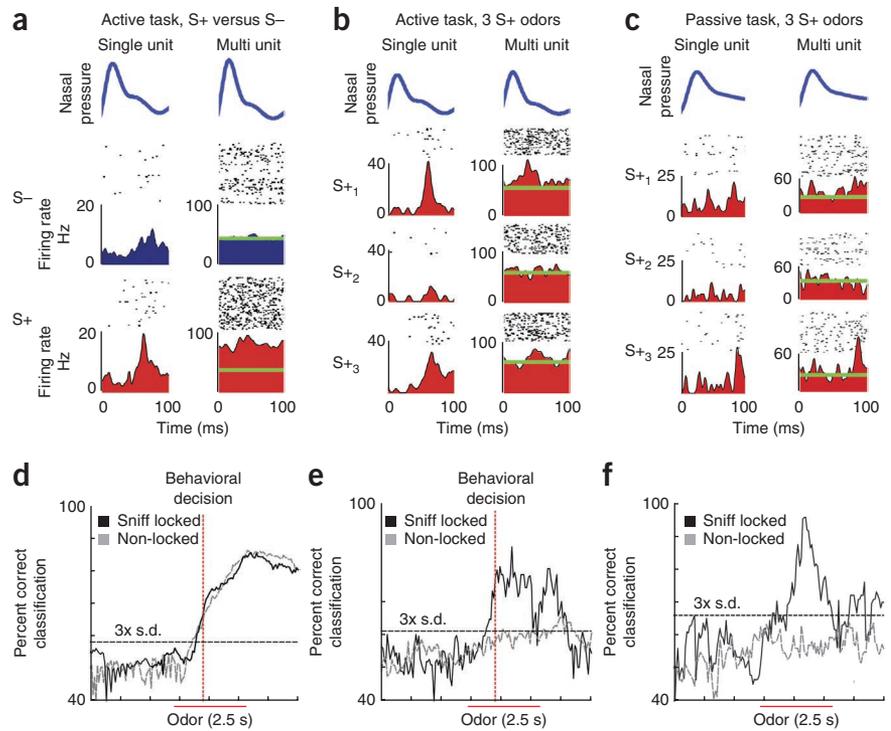


Figure 1 Odor-induced changes in the firing rates of neurons in APC when neuronal firing is not locked to sniffs (sniff onset after odor addition differs from trial to trial). (**a-c**) Active task. (**a**) Examples of odor-induced responses. Left, raster plots. Right, peristimulus histograms for the firing rate (red lines indicate s.e.m.). S-, unrewarded odor; S+, rewarded odor; thick red line, odor exposure; u1, unit 1; u2, unit 2. (**b**) Top, time course for the Euclidean distance between rewarded and unrewarded odors in all experiments. Blue line indicates average and broken red lines indicate s.e.m. Bottom, P value for a rank-sum test of the difference in the Euclidean distance between rewarded and unrewarded responses ($P < 0.05$ at 0.45 s; red line represents $P = 0.05$, 150-ms bins for **a** and **b**). (**c**) Top, time course for licking (1 = licking continuously, mean \pm s.e.m., $n = 20$ sessions). Bottom, P value for a rank-sum test of the difference between licking for rewarded and unrewarded odors ($P < 0.05$ at 0.96 ± 0.1 s, mean \pm s.e.m., $n = 20$). For **b** and **c**, the horizontal black lines indicate odor application and the red and black vertical lines indicate Euclidean distance and licking, respectively. (**d,e**) Histogram for response magnitude (Δz) in responsive multi-units for S+ odors, presented as number of responses divided by number of odors tested per unit.

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Figure 2 Examples showing odor-induced firing in a sniff. (a–c) Top, average sniff pressure transients. Time = 0: transition from exhalation to inhalation. Bottom, raster plots and integrated spike histograms within sniffs. Green lines indicate average firing rate during unrewarded (a,b) or the displayed rewarded odor (c). (d–f) Black trace indicates time course for ideal observer discrimination performance for different odors (S+ versus S– in d and S+₁ versus S+₂ in e and f), calculated using the sniff-locked firing rates from cells recorded in a–c (Online Methods). Red bar indicates odor presentation. Broken gray lines indicate randomizing firing across sniffs eliminates all sniff-locked information; only changes in rate contribute to the discrimination performance (Supplementary Fig. 8). Task conditions: Active task, S+ versus S– (d), active task, S+ odor 1 versus S+ odor 3 (e), and passive task, S+ odor 1 versus S+ odor 3 (f).



to rewarded odors; active task: single unit, 2.8%; multi-unit: 25.4%; passive task: single unit, 0.9%; multi-unit, 7.8%; $P < 0.006$, χ^2 false discovery rate-corrected; responses to unrewarded odor, active task: single unit, 16%; multi-unit, 64%; Fig. 1d,e).

This raises the question of how the olfactory system transmits information on the identity of the odor while monitoring odors passively. Sniffing delivers odors to the olfactory epithelium and studies have found that mitral cells and APC neurons^{5,8–14} can convey information on odor identity by transient firing locked to sniff onset (sniff lock). We found substantial differences in the sniff-locked firing rate of action potentials recorded during the response to different rewarded odors regardless of whether the task was active or passive (Fig. 2a–c and Supplementary Figs. 5–7). To ascertain whether information on the odors is reliably transmitted, we computed the percent correct discrimination of an ideal observer between the different odors on the basis of sniff-locked firing rate from all responsive units during a given experiment. We found clear increases in the ability of an ideal observer to discriminate between odors in both active and passive tasks (Fig. 2d–f). Notably, when comparing reinforced and unreinforced odor responses in the active task, a non-sniff-locked rate code carried most of the information, as there was little difference in performance when we eliminated all sniff-locked information by randomizing the timing of action potentials relative to sniffing (Fig. 2d and Supplementary Fig. 8). This is in stark contrast with discrimination performance among reinforced odors in the active (Fig. 2e) and passive (Fig. 2f) tasks, where there was a clear drop in performance when sniff-locked information was eliminated.

We then analyzed data from the sniff-locked responses of all units in active and passive tasks. The percent of passive task sniff-locked responses of 8.5 and 39% in single and multi-units, respectively, was significantly larger than the non-sniff-locked firing rate responses (single unit: sniff-locked, 8.5%; non-sniff-locked, 0.9%; multi-unit: sniff-locked, 39%; non-sniff-locked, 7.8%; $P < 0.01$, χ^2 ; sniff-locked responses in the active task: single unit, 15.8%; multi-unit, 35%). We then compared the ability of an ideal observer to discriminate between odors before and after randomizing the firing of action potentials to eliminate sniff-locked information (Fig. 3a and Supplementary Figs. 6–8). Randomizing did not alter performance in discriminating

between the reinforced and unreinforced odors in the active task, indicating that discrimination during the active task involves a non-sniff-locked rate code (correlation coefficient = 0.94, $P = 2 \times 10^{-4}$; Fig. 3a). However, randomizing markedly decreased the ability to discriminate between rewarded odors in both the active and passive tasks (active: correlation coefficient = 0.39, $P = 0.01$; passive: correlation coefficient = 0.07, $P = 0.71$; Fig. 3a,b).

We found that information on odor value and identity are multiplexed in APC. On the one hand, information on value is transmitted through changes in firing rate in the active odor detection task, but not in the passive task. On the other hand, information on odor identity is transmitted under both conditions as a change in sniff-locked spiking. Notably, information on value and identity is transferred in parallel, unlike in the taste system, where they are transferred sequentially¹⁵.

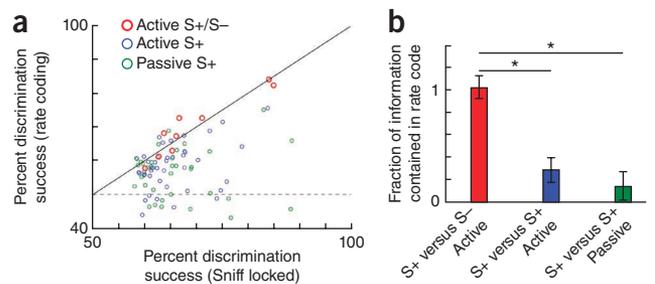


Figure 3 Summary of sniff-locked odor responses. (a) Change in performance of an ideal observer discriminating between the indicated odors (rewarded versus unrewarded, or rewarded versus rewarded) using sniff-locked firing rates or rate coding alone during odor exposure in active and passive tasks. Solid line indicates slope = 1. (b) Fraction of ideal observer performance by rate coding. Significant differences existed (asterisks) between rewarded and unrewarded performance in the active task, and rewarded and rewarded performance in the active ($P = 0.006$) and passive ($P = 0.002$) tasks. Error bars indicate s.e.m.

METHODS

Methods and any associated references are available in the [online version of the paper](#).

Note: Supplementary information is available in the [online version of the paper](#).

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AUTHOR CONTRIBUTIONS

J.D.W. and D.R. formulated the experimental paradigm and designed all the experiments. J.D.W. performed all the experiments and extracted single and multi-units from the raw data. D.H.G. and D.R. performed analysis of the data and generated all the figures. W.D. and D.R. set up the awake behaving recording system and D.R. wrote all programs necessary to run the experiments. W.D. trained J.D.W. on how to perform surgeries and run the experiments with awake behaving mice. All authors had the opportunity to discuss the results, participated in writing and made comments on the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Microarray implantation. To minimize inflammation and cell death, the mice's drinking water was supplemented with minocycline (100 mg l⁻¹) 24 h prior and 72 h after surgery¹⁶. We anesthetized 6 male 8–13-week-old C57BL/6 mice with intraperitoneal injection of ketamine (100 mg per kg of body weight) and xylazine (10 mg per kg) and implanted them with 1 × 8 electrode arrays with 4.8-mm-long electrodes spaced 200 μm apart and coated with parylene C (3–4 MΩ at 1 kHz) (Micro Probe)^{3,17}. Arrays spanned a diagonal in the APC from 1.6 mm anterior to bregma and 2.3 mm from the midline to 0.2 mm anterior to bregma and 3.4 mm from the midline, at depths of 3,060–4,200 μm (mean = 3,625 ± 335 μm, layer II of APC).

Targeting was verified by MRI using a 4.7-T MR animal scanner (Bruker Medical) in mice anesthetized with 1.5–2.5% (vol/vol) inhaled isoflurane and injected intravenously with a Multihance (0.2 mmol kg⁻¹) (**Supplementary Fig. 2**). Data from an array inadvertently inserted in the claustrum rather than the APC was excluded. All animal procedures were performed under a protocol approved by the institutional animal care and use committee of the University of Colorado Anschutz Medical Campus.

Training in the active and passive odor tasks. Under computer control, mice were trained to obtain a water reward in both a passive task, in which the mice obtained the reward for licking on a water spout (passive monitoring) regardless of the odor, and an active go–no go task, in which the mice obtained the water reward only in trials with reinforced odors (no reward for trials with the single unreinforced odor)^{3,17,18}. Please note that, in the passive task, the mouse did move in the chamber actively, but it did not need to perform active detection of the odor on the basis of odor identity to obtain reward.

Passive task. The odor was directed toward the mouse's nose by turning on a final valve and arrived 0.3 s later, as checked with a photoionization detector (mini-PID, Aurora Scientific). To receive the water reward in the passive task, mice had to lick at least once in each 0.5-s interval during a 2-s lick period that occurred 0.5–2.5 s after opening of the final valve (**Supplementary Fig. 1**). If they licked at least once in all four intervals, they received 10 μl of water. Mice received a different odor during each trial, but obtained the water reinforcement regardless of the odor (passive monitoring). Five mice completed this task. Each session included 10–15 pseudorandom trials for each of 8–10 odors. In the passive task, we used 1-octanol, 1-octene, 1-pentanol, 2,5-dimethylpyrazine, air, decanal caprinaldehyde, ethyl vanillin, female bedding, ferret, geraniol, methyl benzoate, myrcene, 2-nonanone, octyl aldehyde, pentadecane, propionic acid, propyl acetate, tert-amyl alcohol and tetradecane as odors (also used as reinforced odors in the go–no go task).

Active odor monitoring task. The active go–no go task was similar to the passive task, with the addition of unrewarded trials. In the go trials, mice were exposed to one of five or six of the odors listed above, for which they received a water reward if they licked correctly (reinforced odors). In the no-go trials, they were exposed to one unreinforced odor (1% cuminaldehyde). Mice did not receive water on no-go trials, regardless of whether they licked or not. Given that mice prefer not to expend energy on licking in the unrewarded trials, it was advantageous to them to pay attention to rewarded versus unrewarded odors in this task. Six mice completed this task. Each session included 50–60 unrewarded trials and 10–15 trials for each rewarded odor. Rewarded and unrewarded odors were pseudorandomly interspersed in each block of 20 trials. Given that the mouse behavioral setup was under computer control, it was not necessary for the experimenter to be blind to the trial conditions.

Recording setup and offline spike clustering. Output of the two electrode arrays was monitored and digitized as in previous studies^{3,17}. Waveforms were thresholded and clustered for similar shape by wavelet decomposition and superparamagnetic clustering^{2,5}. A single unit was defined using the criterion of finding <3% of the spikes in the refractory period of 2 ms (**Supplementary Fig. 3**).

Analysis of odor-elicited changes in firing rate. Analysis for odor-induced changes in the rate of firing of neurons when neuronal firing is not locked to sniffs (sniff onset after odor addition differs from trial to trial; **Fig. 1**) was performed

using MATLAB programs tested using simulated data^{3,17}. Briefly, each go–no go session typically included 50 trials with the unrewarded odor and 15 trials with each of the four or five rewarded odors. Responsiveness was determined by a *t* test of the odor-induced changes in firing rates compared between 2 s before odor application and 2 s following odor presentation. In each experiment, the *P* values were corrected for multiple comparisons using the false discovery rate¹⁹, a statistical method previously used by our group¹⁷ that is suitable for testing significant differences in large data sets and does not require independent data^{20,21}. No statistical methods were used to pre-determine sample sizes, but our sample sizes are similar to those reported in previous publications³.

Results were reported on 216 multi-units and 139 single units in 27 active odor discrimination experiments. To compare firing rate divergence (calculated using Euclidean distance) with divergence of licking for rewarded and unrewarded odors as a function of time during the trial, we used 20 of the 27 experiments in which we recorded both licking and multi-electrode array voltage (196 multi-units and 126 single units). Results are reported for 176 multi-units and 70 single units in 21 passive experiments.

Analysis of odor-elicited changes in sniff-locked action potential firing in a sniff. Sniffs and unit activity were simultaneously recorded in three mice with surgical cannula implantation³. Sniffing was monitored by recording intranasal pressure through the implanted nasal cannula connected to a pressure sensor (model no. 24PCEFA6G(EA), 0–0.5 psi, Honeywell) via polyethylene tubing²² mounted on a commutator (Tucker Davis Technologies). Pressure transients were digitized at 24 kHz. To detect a sniff, we set a positive threshold and detected each sniff as occurring at the point at which the pressure signal exceeded threshold (the transition from exhalation to inhalation). Spikes were collected for each sniff from 10 ms before to 100 ms after the onset of inhalation.

To analyze the effect of temporal coding relative to sniffing, we collected spikes for each sniff with 1-ms resolution (110 points), with 1 corresponding to the occurrence of a spike and 0 to no spike. Each array was convolved with a Gaussian ($\sigma = 5$ ms). Following convolution, all sniffs were compiled into a matrix and weighted to make the average zero and to transform variance to unit. Spiking from each unit during each sniff was therefore represented by a point in 110-dimensional space. Consistent with previous findings¹⁰, we found that information was contained in the sniff-locked spike rate and not in the phase of spikes relative to the sniff (**Supplementary Figs. 6 and 7**). Thus, for subsequent analyses, our technique was simplified to only include the spike rate locked to each sniff for each unit.

Sniff-locked firing rates were used as the input to perform ideal observer analysis (**Figs. 2 and 3** and **Supplementary Figs. 5–8**). In the analysis shown in **Figure 2**, the sniff-locked firing rates of all responsive units recorded during that experiment were compiled into a vector for each sniff. A sliding window (500 ms) was used to select which sniffs to include in the analysis at each time point for the time courses shown in **Figure 2d–f**. A template-matching algorithm¹² was used to calculate discrimination performance between odors. This measure was repeated for every odor combination in every time bin for each experiment, yielding time courses for ideal observer discrimination performance. For the data shown in **Figure 3**, the same procedure was followed with the input to the classifier in this case being the sniff-locked rates from individual units.

The sniff-locked firing behavior is reported for the following number of pairs of odors: 212 multi-unit and 79 single unit odor pairs comparing rewarded versus unrewarded trials in five active odor discrimination experiments, 38 multi-unit and 12 single unit odor pairs in rewarded versus unrewarded trials in the five active odor discrimination experiments, and 180 multi-unit and 50 single unit odor pairs in seven passive odor monitoring experiments.

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