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Information for Decision Making and Stimulus Identification is Multiplexed in Sensory Cortex

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Abstract

In recordings from anterior piriform cortex (APC) in awake behaving mice we find that neuronal firing early in the olfactory pathway simultaneously conveys 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 receptors[1] into odor value after one or two synapses[2,3]. Synchronous firing of mitral cells then transfers information to the cortex[3–5]. The early olfactory system thus faces the challenge of transmitting information about both stimulus “value” vs. “identity”. Multiplexing is one mechanism to simultaneously transmit this information[6], but how information on odor value and identity is multiplexed is not understood.

In humans, APC responds to olfactory stimuli when actively detecting odors, but shows reduced fMRI signals when the subject is passive[7]. Yet, even in passive sampling, odor identity is conveyed to detect deleterious odors. Here we ask whether transmission of information regarding identity and value through APC is multiplexed, and whether coding differs during active vs. passive monitoring.

In the active odor detection task the mouse received water for licking when presented rewarded odors, and not when exposed to unrewarded odor (Fig. 1, Supplementary Figs. 1–4). They responded correctly in 87±6% of trials (±SEM, n=20). As expected[4,5], odors elicited changes in firing rate that differed between rewarded and unrewarded (Fig. 1). A

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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.

substantial number of units responded to odor with altered firing rate (22% of 139 single and 63% of 216 multi units). Consistent with a study in mitral cells[3] this indicates that firing rate conveys information on odor value. To determine when changes in firing rate of APC cells convey sufficient information we generated the Euclidean distance between rewarded and unrewarded in multidimensional space with odor-elicited changes in firing rate for APC cells in each dimension. Euclidean distance increased substantially prior to the behavioral decision time (Fig. 1c).

Odors elicit substantially reduced fMRI signals in passive tasks in humans[7] raising the question whether odor-induced changes in firing rate of APC neurons decrease in passive tasks. We tested APC neuron responses during a passive task where mice did not actively discriminate between odors (rewarded for licking for *any* odor). Consistent with human studies[7], the data show substantially reduced responses to rewarded (S+) odors in the passive task (Fig. 1d,e). Responses to S+ odors: active task 2.8 and 25.4% in single and multi units and passive task 0.9 and 7.8% respectively ($p<0.006$, Chi Squared FDR-corrected; for the active task S− odor 16% and 64% responsive single and multi units).

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 show 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 significant differences in sniff-locked firing rate of action potentials recorded during the response to different S+ odors regardless whether the task is active or passive (Fig. 2a–c and Supplementary Fig. 5–7). In order to ascertain whether information on the odors is reliably transmitted we computed the percent correct discrimination of an ideal observer between the different odors based on 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. 2 d–f). Importantly, 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 (in Fig. 2d dashed lines, see Supplementary Figure 8). This is in stark contrast to 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 (solid vs. dotted lines, Fig. 2 e and f).

Next we 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 non-sniff locked firing rate responses: 0.9 and 7.8% respectively (Chi-Squared $p<0.01$, the percent of units with sniff-locked responses in the active task was 15.8 and 35% in single and multi units). 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 see 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 (Fig. 3a, red circles, correlation coefficient –corrc

0.94, p=2 10^{-4}). However, randomizing did dramatically decrease the ability to discriminate between rewarded odors in both the active and passive tasks (Active: Fig. 3a, blue circles, corrc 0.39, p=0.01, and passive, green circles, corrc=0.07, p=0.71).

We have shown that information on odor value and identity are multiplexed in APC. 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 sequentially[15].

METHODS

Microarray Implantation

To minimize inflammation and cell death animals' drinking water was supplemented with minocycline (100 mg/L) 24 hours prior and 72 hours after surgery[16]. Six male8-to 13-week-old C57BL/6 mice were anesthetized with intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg) and implanted with 1×8 electrode arrays with 4.8 mm long electrodes spaced 200 μm apart coated with parylene C (3–4 MΩ at 1 kHz) (Micro Probe, Inc., Gaithersburg, MD)[3,17]. Arrays spanned a diagonal in the anterior piriform cortex (APC) from 1.6 mm anterior to bregma, 2. 3 mm from the midline to 0.2 mm anterior to bregma, 3.4 mm from the midline at depths ranging from 3060 to 4200 mm (mean=3625±335 mm, layer II of APC).

Targeting was verified by magnetic resonance imaging (MRI) using a 4.7 Tesla MR animal scanner (Bruker Medical, Billerica, MA) in mice anesthetized with 1.5 –2.5% inhaled isoflourane and injected intravenously with a Multihance (0.2 mmol/kg) (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.

Training in the active and passive odor tasks

Under computer-control mice were trained to obtain a water reward in both a "passive" task, where regardless of the odor they obtained the reward for licking on a water spout (passive monitoring), and an "active" go-no go task in which they 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 animal does move in the chamber actively, but it does not need to perform active detection of the odor based on odor identity to obtain reward.

Passive task—The odor was directed towards the mouse's nose by turning on a final valve (FV), and arrived 0.3 sec later as checked with a photoionization detector (mini-PID; Aurora Scientific Inc., Aurora, ON, Canada). To receive the water reward in the passive task, mice had to lick at least once in each 0.5 sec interval during a 2 sec lick period that took place from 0.5 to 2.5 sec after opening of the FV (see Supplemental Fig. 1). If they

licked at least once in all 4 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 animals completed this task. Each session included 10–15 pseudorandom trials for each of 8–10 odors. Odors used in the passive task (also used as reinforced odors in the go-no go task): 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, tetradecane.

Active odor monitoring task—The active go-no go task was like the passive task, with the addition of unrewarded trials (S−). In the go trials, mice were exposed to one of 5–6 of the odors listed above for which they received a water reward if they licked correctly (reinforced S+ odors). In the no-go trials they were exposed to one unreinforced (S−) odor (1% cumin aldehyde). Mice did not receive water on no-go trials regardless of whether they licked or not. Since animals prefer not to expend energy on licking in the S− trials, it was advantageous to them to pay attention to S+ vs. S− odor category in this task. Six animals completed this task. Each session included 50–60 unrewarded trials and 10–15 trials for each rewarded odor. S+ and S− odors were pseudorandomly interspersed in each block of 20 trials. Since 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 in 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 included typically 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 seconds before odor application and 2 seconds following odor presentation. Within each experiment, the p values were corrected for multiple comparisons using the false discovery rate[19], a statistical method previously used by our group[17] 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 publications[3].

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 where we recorded both licking and

multielectrode 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 within a Sniff

Sniffs and unit activity were simultaneously recorded in three mice with surgical cannula implantation[3]. 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, Canada) via polyethylene tubing[22] mounted on a commutator (TDT: Tucker Davis Technologies, Alachua, FL). Pressure transients were digitized at 24 kHz. To detect a sniff a positive threshold was set and each sniff was detected as occurring at the point where 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 impact of temporal coding relative to sniffing, spikes were collected for each sniff with 1 ms resolution (110 points) with 1 corresponding to the occurrence of a spike and 0 no spike. Each array was convolved with a Gaussian (σ= 5 ms). Following convolution, all sniffs were compiled into a matrix and weighted to make the average zero and transform variance to unit. Spiking from each unit during each sniff was thus represented by a point in 110-dimensional space. Consistent with a study of Miura and co-workers[10] 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 Supplemental 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 Fig. 2d, e and f. A template-matching algorithm[12] 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 S+ vs. S+ trials in five active odor discrimination experiments, 38 multi unit and 12 single unit odor pairs in S+ vs. S− trials in the five active odor discrimination experiments and 180 multi unit and 50 single unit odor pairs in seven passive odor monitoring experiments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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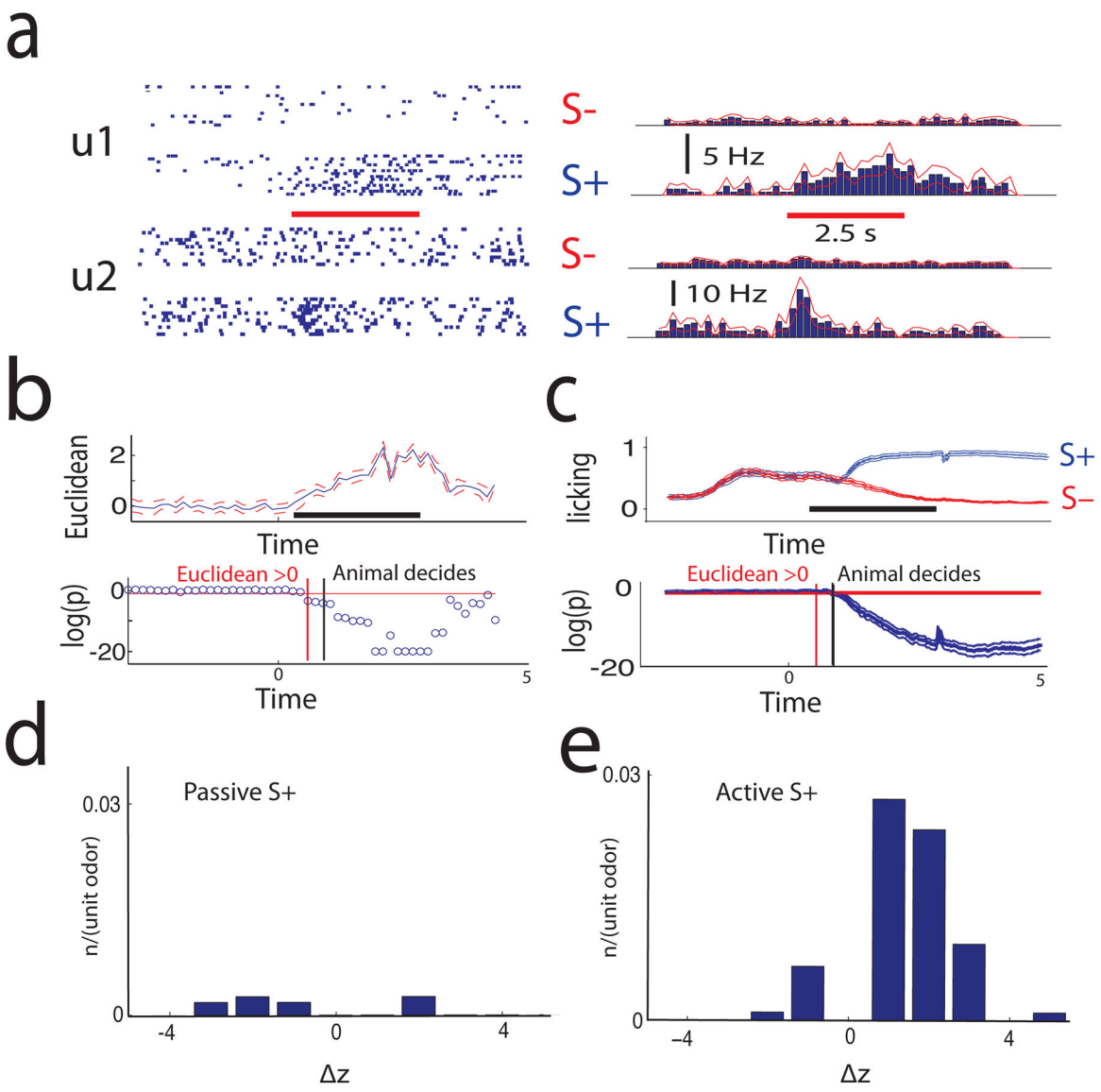

Figure 1.

Odor-induced changes in firing rate of neurons in APC when neuronal firing is not locked to sniffs (sniff onset after odor addition differs from trial to trial). **a** to **c:** active task. **a.** Examples of odor-induced responses. Left: raster plots, right: peristimulus histograms for the rate of firing (red lines: s.e.m.). S−: unrewarded, S+: rewarded odor; red line: odor exposure. u1, u2: units 1 and 2. **b.** Top: Time course for the Euclidean distance between rewarded and unrewarded odors in all experiments. Blue line: average, broken red lines: s.e.m. Bottom: p-value for a ranksum test of the difference in the Euclidean distance between rewarded and unrewarded responses. p-value<0.05 at 0.45 sec; red line p=0.05 (150 msec bins for **a** and **b**) **c**. Top: Time course for licking (1 = licking continuously, mean ±s.e.m. n= 20). Bottom: p-value for a ranksum test of the difference between licking for rewarded and unrewarded odors (p<0.05 at 0.96+0.1 sec, mean+s.e.m., n=20). blue: reinforced, red: unreinforced. **b** and **c**: horizontal black lines: odor applied; vertical lines: red

(Euclidean distance), black (licking). **d, e**. Histogram for response magnitude (Δz) in responsive multi units for S+ odors (**d**: passive, **e**: active).

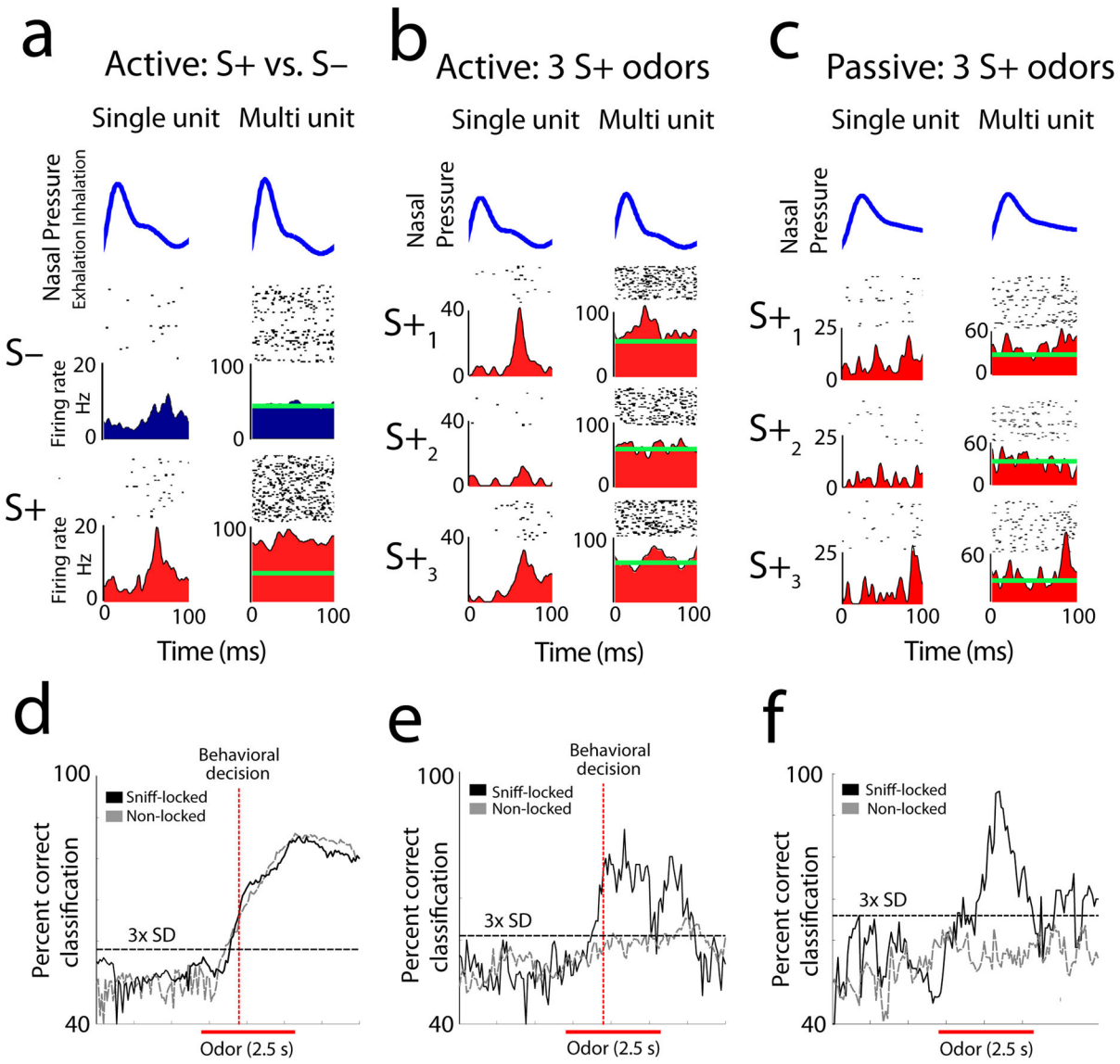

Figure 2.

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

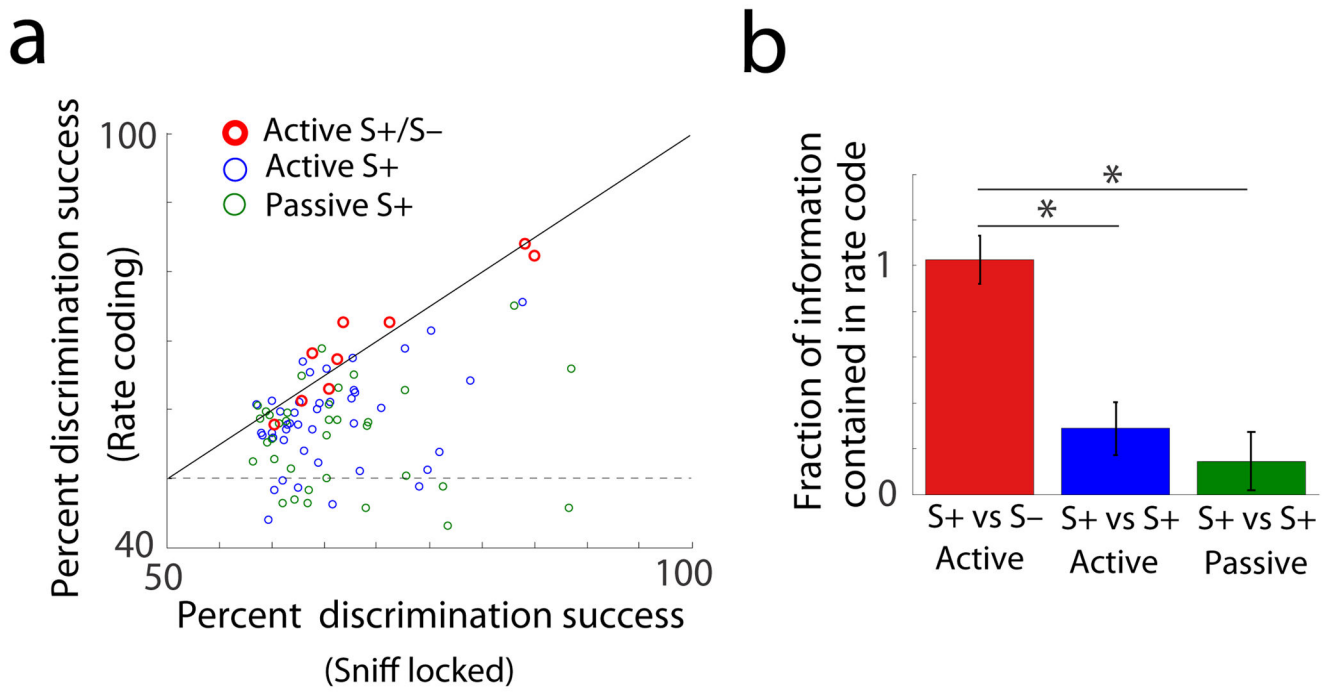

Figure 3.

Summary of sniff-locked odor responses. **a.** Change in performance of an ideal observer discriminating between the indicated odors (S+ vs S− or S+ vs S+) using sniff-locked firing rates or rate coding alone during odor exposure in active and passive tasks. Solid line: slope=1. **b.** Fraction of ideal observer performance by rate coding. Significant differences exist (asterisks) between S+ vs. S− performance in the active task and S+ vs. S+ performance in the active ($p = 0.006$) and passive ($p = 0.002$) tasks. Bars indicate s.e.m.