

Sensory and Motor Systems

Prenatal and Early Postnatal Odorant Exposure Heightens Odor-Evoked Mitral Cell Responses in the Mouse Olfactory Bulb

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Abstract

Early sensory experience shapes the anatomy and function of sensory circuits. In the mouse olfactory bulb (OB), prenatal and early postnatal odorant exposure through odorized food (food/odorant pairing) not only increases the volume of activated glomeruli but also increases the number of mitral and tufted cells (M/TCs) connected to activated glomeruli. Given the importance of M/TCs in OB output and in mediating lateral inhibitory networks, increasing the number of M/TCs connected to a single glomerulus may significantly change odorant representation by increasing the total output of that glomerulus and/or by increasing the strength of lateral inhibition mediated by cells connected to the affected glomerulus. Here, we seek to understand the functional impact of this long-term odorant exposure paradigm on the population activity of mitral cells (MCs). We use viral expression of GCaMP6s to examine odor-evoked responses of MCs following prenatal and early postnatal odorant exposure to two dissimilar odorants, methyl salicylate (MS) and hexanal, which are both strong activators of glomeruli on the dorsal OB surface. Previous work suggests that odor familiarity may decrease odor-evoked MC response in rodents. However, we find that early food-based odorant exposure significantly changes MC responses in an unexpected way, resulting in broad increases in the amplitude, number, and reliability of excitatory MC responses across the dorsal OB.

Key words: mitral cells; olfaction; olfactory bulb; plasticity

Significance Statement

The structure and output of the olfactory bulb (OB) circuit can be modified by odor experience throughout both development and adulthood. The highly specific organization of this system lends itself to detailed analyses of how experience can shape the architecture of sensory system responses. Previous work demonstrated that prenatal and early postnatal odorant exposure using a food-based paradigm increased the number of primary projection neurons connected to activated glomeruli. This increase may have significant effects on odorant representation and OB output. In this study, we focus on understanding how this odorant exposure paradigm impacts the odor-evoked responses of one population of primary OB output neurons, the mitral cells (MCs).

Introduction

The olfactory bulb (OB) has a stereotyped structure, the organization of which is dictated in part by odorant receptor (OR) identity (Ressler et al., 1994; Vassar et al., 1994;

Potter et al., 2001; Bozza et al., 2002; Treloar et al., 2002; Feinstein and Mombaerts, 2004; Komiyama and Luo, 2006). Olfactory sensory neurons (OSNs) send axons to

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the OB, where axons from OSNs expressing the same OR converge into roughly spherical structures called glomeruli. Each glomerulus contains dendrites of a cohort of juxtglomerular and primary projection neurons, the mitral and tufted cells (M/TCs), together, these make up a glomerular module, the basic odor-coding unit of the OB. Each glomerular module thus has a genetic identity based on OR expression as well as a cohort of activating odorant ligands for the corresponding OR.

This OR identity-based organization facilitates the investigation of how specific olfactory experiences change the OB. Anatomic studies demonstrate that the OB circuitry is highly plastic and subject to experience-dependent structural changes throughout both development and adulthood. Odor exposure using a number of different conditioning paradigms increases glomerular volume (Woo et al., 1987; Todrank et al., 2011; Dias and Ressler, 2014; Morrison et al., 2015). Combined prenatal and early postnatal odor exposure increases both glomerular volume and associated M/TC number (Todrank et al., 2011; Liu et al., 2016), while early aversive conditioning accelerates the rate of glomerular convergence (Kerr and Beluscio, 2006). Early postnatal passive odorant exposure has also been shown to decrease cell turnover in the glomerular and granule cell layers (Woo et al., 2006). In adult mice, aversive odorant conditioning increases OSN number and glomerular volume, these changes are reversed following the extinguishing of the learned aversive response (Morrison et al., 2015). These studies use different conditioning or exposure paradigms, yet they all have significant effects of OB circuitry, suggesting that several distinct mechanisms may influence the development and maintenance of OB structure. These large modifications to OB circuitry may in turn have significant effects on the representation and processing of odorants in the OB.

Odor-evoked activity in the rodent OB is also influenced by odorant experience and exposure. Rodents demonstrate both acute and chronic reductions in the amplitude and probability of odor-evoked excitatory M/TC responses following repeated odor presentation (Chaudhury et al., 2010; Kato et al., 2012). In rats, early postnatal odorant exposure paired with positive somatosensory stimuli decreases the number of excitatory M/TC responses elicited by the learned odorant while leaving responses to unassociated odorants unchanged (Wilson et al., 1985, 1987). However, the relationship between experience-dependent anatomic circuit changes and odor-evoked activity in the OB is not well understood, as there is significant interstudy variation in the delivery, context, and timing of odorant exposure or conditioning.

Here, we use an odor exposure paradigm with a previously characterized anatomic correlate to understand

how odor representation may be affected by these changes in circuit structure. Todrank et al. (2011) and Liu et al. (2016) found that food-based prenatal and early postnatal odorant exposure increases glomerular volume and the number of M/TCs corresponding to activated glomerular modules, while increasing behavioral preference for the odor that was paired with food. Given that a single odorant activates multiple glomeruli across the OB, these observed changes in glomerular volume and M/TC number could be generalized to many glomeruli and thus have a large distributed impact on odor representation in the OB. We investigate how early chronic food-based odorant exposure, a paradigm known to change OB anatomy, impacts the odor-evoked responses of MCs in the dorsal mouse OB. Surprisingly, we find that odor-evoked calcium transients in MCs are broadly enhanced rather than reduced in this food pairing paradigm.

Materials and Methods

All animal procedures were performed in accordance with the University of Pittsburgh animal care committee's regulations.

Animals and surgical methods

All experiments were done in male and female M72-IRES-tauGFP mice. Imaging was done on adult mice, at five to eight weeks of age. During all surgical procedures, animals were anesthetized using a ketamine/xylazine mixture, provided analgesia using carprofen injections, and maintained at 37°C body temperature. Expression of GCaMP6s was achieved through injection of 1 μ l of AAV-hsyn-GCaMP6s \sim 250 μ m underneath the pial surface in the dorsal posterior OB surface. Animals were allowed to recover for two weeks, after which they were used for acute *in vivo* anesthetized imaging experiments. For acute imaging, a 2-mm diameter craniotomy was made over the dorsal posterior OB and covered with low melting point agarose. A coverslip was secured over the craniotomy using dental cement to minimize z-plane movement. Imaging was done in anesthetized animals maintained at 37°C body temperature.

Odorant exposure

Prenatal and early postnatal odorant exposure was performed on M72-IRES-tauGFP mice as detailed in Todrank et al. (2011) and Liu et al. (2016). Two qualitatively distinct odorants were used for odor-exposure groups. Methyl salicylate (MS) has a wintergreen scent and activates both olfactory and trigeminal responses, while hexanal has a green grass scent and activates only olfactory receptors. Both odorants are strong activators of glomeruli on the dorsal OB surface in rats and mice, with different but overlapping activation areas (Glomerular Activity Response Archive, Michael Leon, gara.bio.uci.edu/index.jsp; Wachowiak and Cohen, 2001, 2003). We exposed animals to these odors by adding these odors to the food provided to these animals, as described previously (Todrank et al., 2011; Liu et al., 2016), a manipulation that results in altered preference for the conditioned food and altered glomerular module structure.

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Food was mixed with either MS (mint) or hexanal (1% by volume) and dried under a fume hood for 3 d. Breeding pairs were fed with either control, mint-scented, or hexanal-scented food for the duration of gestation and nursing (Fig. 1A). Litters were subsequently weaned onto and continuously fed with either control, mint-scented, or hexanal-scented food until acute calcium imaging experiments. Both male and female mice were used for imaging. Breeding pairs were weighed regularly to ensure that odorized food did not interfere with food consumption. Odorant-exposed litters were not of substantially different weights from control litters.

Stimulus delivery

Each trial consisted of 4-s room air, 1-s odorized air, and 25-s room air. Each odorant was presented four times in pseudorandom stimulus order and with intertrial interval length of at least 1 min. Stimuli consisted of 14 odorants: eight odorants diluted in mineral oil at different concentrations [1%: isoamyl acetate (IAA), hexanal, MS, ethyl butyrate (EB), propionic acid (PA), hexanone, acetophenone (AP), and 2-OH acetophenone (THA); 5%: IAA, hexanal, MS; 10%: IAA, hexanal, MS]. All odorants are known dorsal OB ligands. Odorants were delivered using a custom built olfactometer controlled via TTL input from a HEKA ITC-18 external DA/AD/TTL device run by IGOR Pro (RRID:SCR_000325; Fig. 1B).

Imaging

Two-photon imaging was done in the MC layer \sim 150–225 μ m under the pial surface using a VIVO 2-Photon system from 3I Intelligent Imaging Innovations and SlideBook Imaging software. Image capture rate was 6–9 Hz.

Analysis

The dataset consists of 863 putative mitral cells (MCs) from 17 mice (225 cells from one female and four male control mice, 269 cells from two female and four male mint-exposed mice, and 369 cells from two female and four male hexanal exposed) for a total of 12,082 cell-odor pairs. To select regions of interest (ROIs), cell somata in each field of view were traced manually using SlideBook software and avoiding intersecting cell processes as determined by Z-stacks of each field of view (Fig. 1C). Presence or lack of odor-evoked response was not taken into account when identifying cells. Rather, all identified fluorescent cells were included. Raw intensity values were calculated using SlideBook software. $\Delta F/F$ traces were calculated using the average baseline intensity from the 20 frames before stimulus onset at 4 s after start of the trial. Traces were detrended by subtracting a polynomial fit from the calculated $\Delta F/F$ trace (Fig. 1D). We fit the 20 frames before odor onset (\sim 3 s before odor onset) and the last 45 frames (\sim 19 s after odor end) of each trace to a second degree polynomial and subtracted this fit from each trace. Response threshold was set at 3 SD away from baseline. Responses were only counted as successful if above this threshold, otherwise response was recorded as 0. All analysis and visualization was done using Python (RRID:SCR_008394). Some statistical tests were performed using GraphPad Prism (RRID:SCR_002798).

Results

Majority of MC odor-evoked responses were excitatory

MC odor-evoked responses were dominated by excitatory responses, constituting 11,089 cell-odor pairs out of 12,082 total cell-odor pairs or \sim 92% of all measured responses. Both peak $\Delta F/F$ (peak amplitude of $\Delta F/F$ response) and integral of odor-evoked response (integrated $\Delta F/F$) were calculated. A strong correlation between the peak and integral of individual responses was observed (Pearson's $r = 0.97$; Fig. 2C), but there was no significant correlation between initial baseline intensity and either the peak (Pearson's $r = -0.0048$) or integral of responses (Pearson's $r = -0.018$; Fig. 2A,B). Thus, all data were pooled regardless of initial baseline intensity. Given the strong correlation between the peak and integral of odor-evoked response, subsequent data regarding odorant-evoked responses are shown as peak values. The distribution of all responses to all odorants are visualized in Figure 2D, with significant statistical differences observed between the response density functions of MCs from control, hexanal-exposed, and mint-exposed animals (Kruskal–Wallis test with Tukey's multiple comparisons test; $p = 6.65E-199$; adjusted p values between groups approximated 0).

We observe significant cell population differences in response between the control and each of the odor-exposed groups. We next assessed potential differences in odor-evoked response based on subject sex. When examining only male mice (Fig. 2E), we find that the response distributions are different between hexanal-exposed and control mice (Kruskal–Wallis, $p = 1.13E-66$), but not different between mint-exposed and control mice (Kruskal–Wallis, $p = 0.69$). However, when examining cell responses from female mice (Fig. 2F), we find that the distributions of cell responses from both groups of odor-exposed animals significantly differ from the distribution of control mouse responses (Kruskal–Wallis; control vs hexanal exposed: $p = 1.29E-97$; control vs mint exposed: $p = 1.02E-81$). The sample size of female mice ($n = 1$ control, two mint-exposed and two hexanal-exposed female mice) was not sufficient to draw specific conclusions about the role of sex in amplifying odor-evoked responses following early odorant exposure. Given the small animal number, we cannot determine if these intergroup differences are due to interanimal variability or sexual dimorphism. Male and female mice pooled across groups did not have significantly different ages (Student's t test; male vs female; $n = 12$ vs 5; mean $P48.67 \pm 6.56$ vs $P41.8 \pm 9.24$; $p = 0.57$).

Amplitude of excitatory response increases following early odorant exposure

Several additional features of the odor-evoked response change significantly following early odorant exposure, namely amplitude, number, and reliability of response. The amplitude of excitatory odor-evoked responses increased significantly for cells in both odor-exposed groups as compared to cells in the control animals (Fig. 3A). Figure 3B shows clear differences in

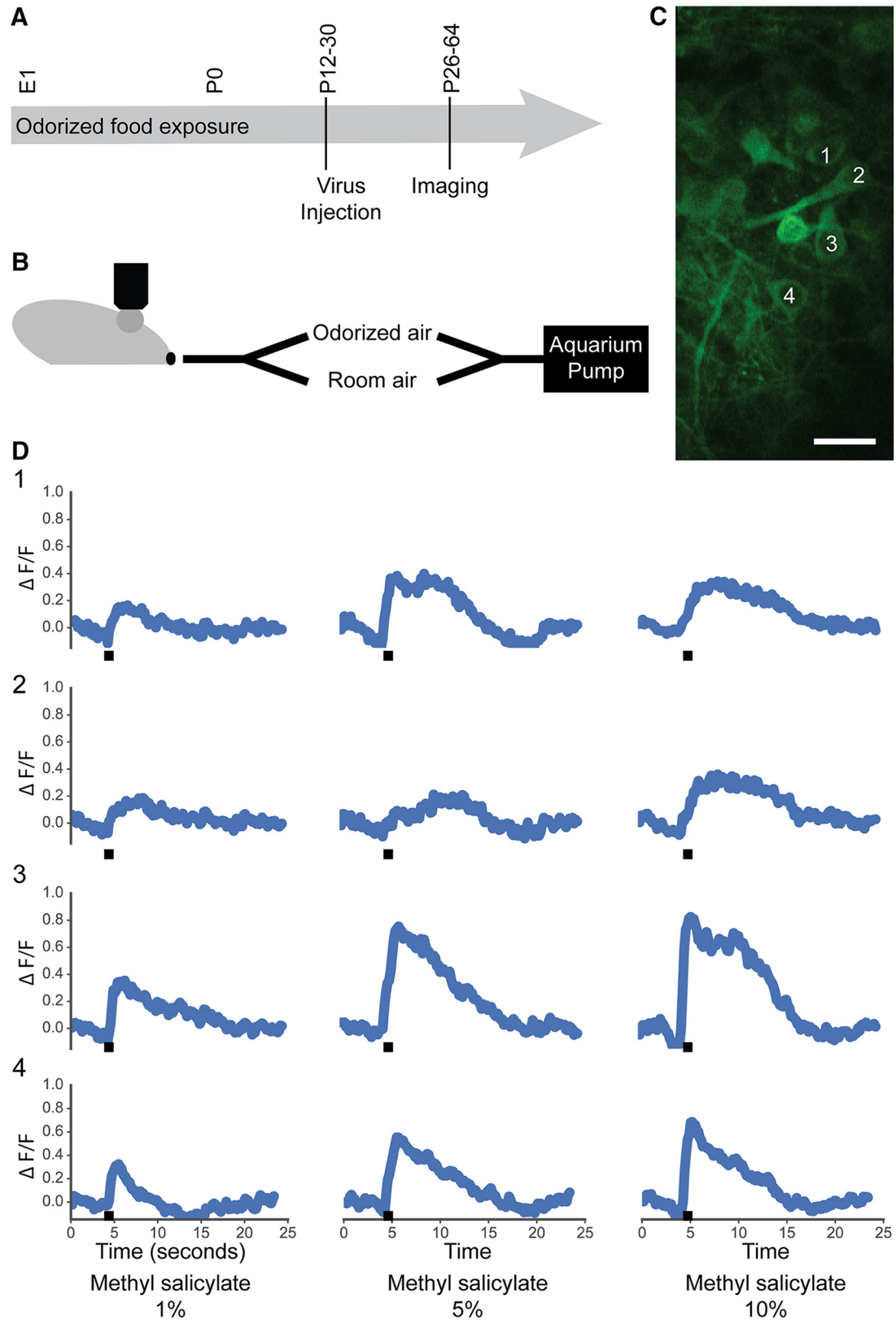


Figure 1. Odor-evoked calcium responses in the MC layer. **A**, Odorized food exposure lasted through the entirety of gestation and the postnatal period until imaging. Virus injection into the dorsal OB was done at P12-P30. Imaging was performed two to three weeks after virus injection. **B**, The dorsal OB was imaged during stimulus presentation using a custom-built 2-channel olfactometer with airflow provided by an aquarium pump. **C**, The MC layer was imaged with manual ROI selection. Four cells are labeled, with corresponding odor-evoked responses shown in **D**. White scale bar: 25 μ m. Black bar indicates 1-s odor stimulus.

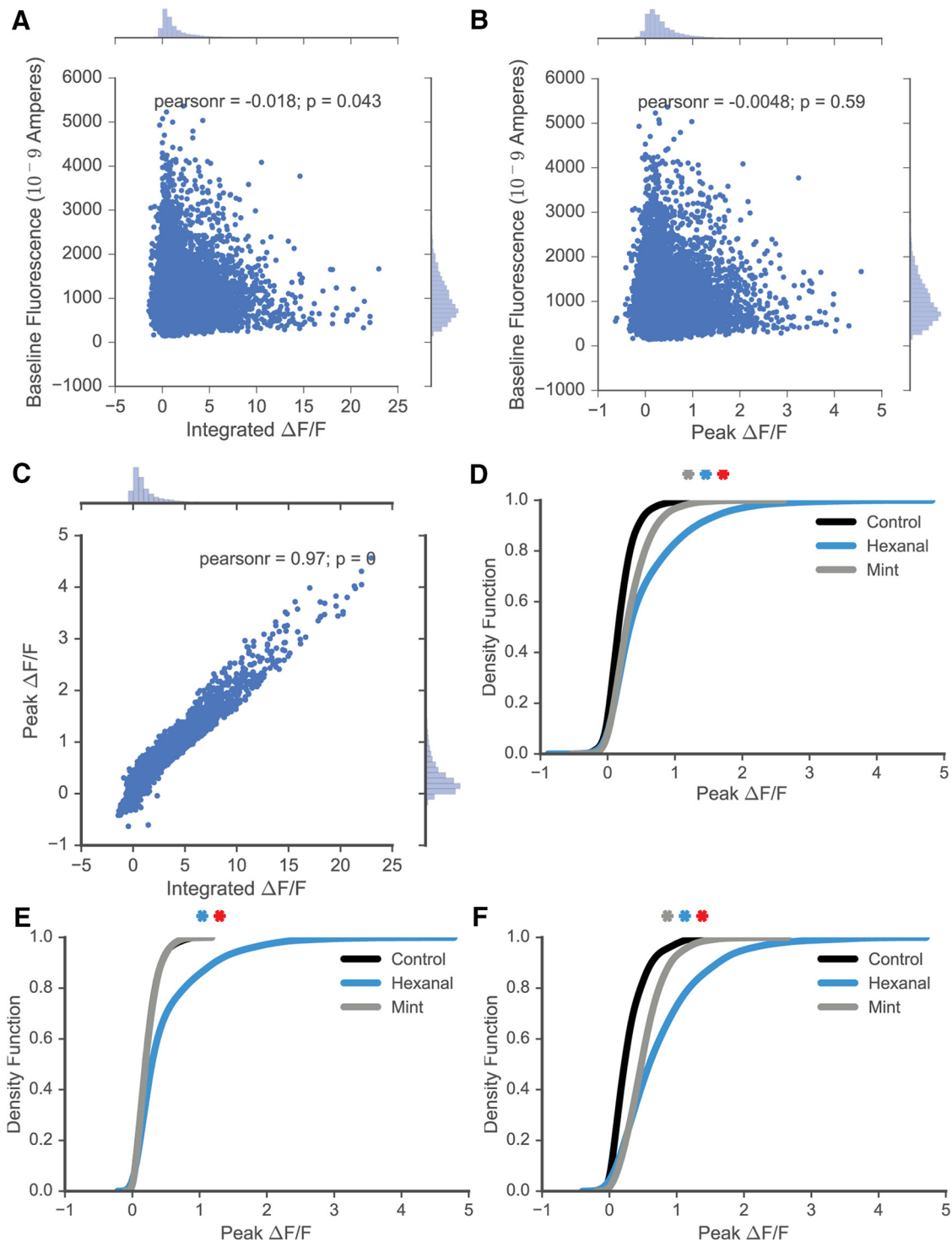


Figure 2. Response characteristics. **A**, Integrated $\Delta F/F$ and baseline fluorescence are not correlated. **B**, Peak $\Delta F/F$ and baseline fluorescence are not correlated. **C**, Peak and integrated $\Delta F/F$ are strongly correlated. **D**, Kernel density estimation (KDE) describing response distribution across all cells and odorants in control, hexanal-exposed, and mint-exposed mice (statistically significant distributions between groups). **E**, KDE describing response distribution across cells in control, hexanal-exposed, and mint-exposed male mice. **F**, KDE describing response distribution across cells in control, hexanal-exposed, and mint-exposed female mice. Gray asterisk, statistically significant difference between mint and control groups; blue asterisk, statistically significant difference between hexanal and control; red asterisk, statistically significant difference between hexanal and mint.

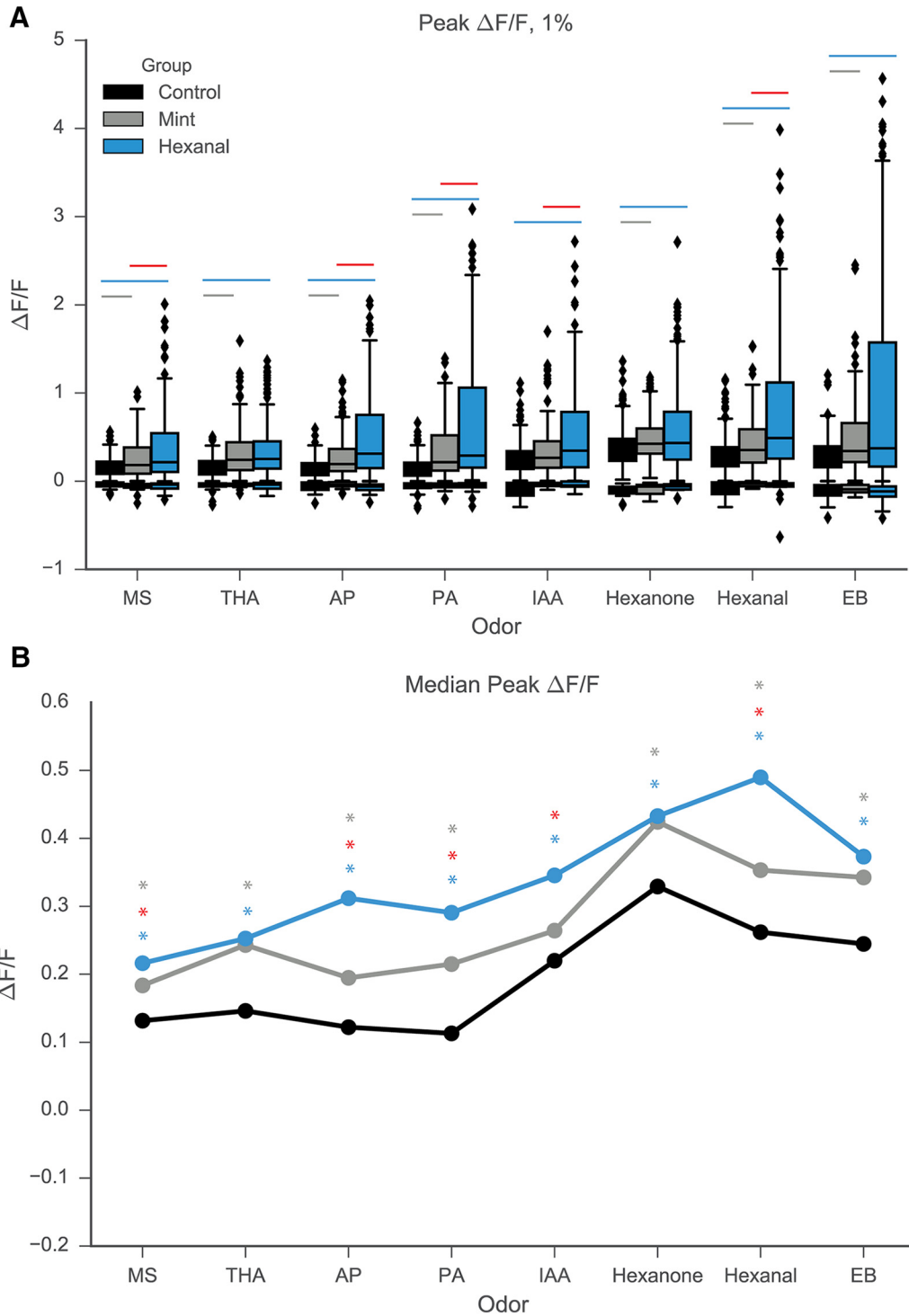


Figure 3. Odor exposure increases median Peak $\Delta F/F$ of MC response to odorants at 1% concentration by volume. **A**, Boxplot describing distribution of peak odor-evoked $\Delta F/F$ of MC response to odorants at 1% concentration by volume. **B**, Median peak $\Delta F/F$ across all odorants at 1% concentration by volume. Gray asterisk, statistically significant difference between mint and control groups; blue asterisk, statistically significant difference between hexanal and control; red asterisk, statistically significant difference between hexanal and mint.

median $\Delta F/F$ across all odorants at 1% concentration for control versus hexanal- and mint-exposed and for hexanal versus mint-exposed. This increase in response amplitude is not odor-specific, but rather is

observed for almost all odors and concentrations in the odor stimulus panel (Figs. 3, 4). Descriptive and odor-specific group comparison statistics can be found in Table 1.

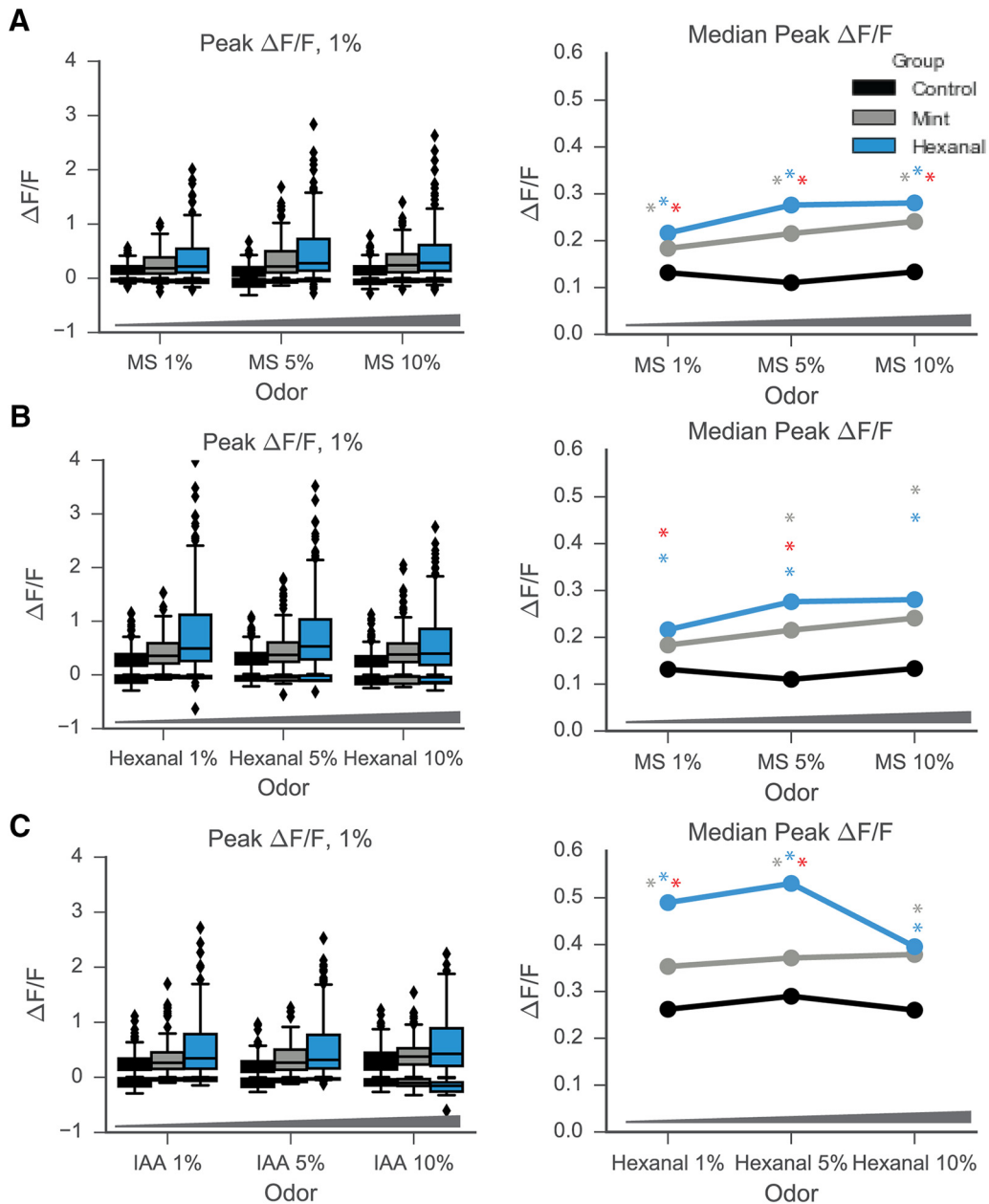


Figure 4. Odor exposure increases median Peak $\Delta F/F$ of MC response to odorants across concentrations. Mint-exposed and hexanal-exposed MCs have higher medians of peak $\Delta F/F$ across all concentrations of MS (**A**), IAA (**B**), and hexanal (**C**). There were no significant differences in response distribution between concentrations for any exposure group.

Within-group differences in responses to the same odorant were also observed across concentrations (Fig. 4). However, these concentration differences were largely similar between the control, mint-exposed, and hexanal-exposed groups, suggesting that the increased responsiveness seen following odor exposure was not specific to a single concentration. There were two combinations of odorants and concentrations for which significant differences were observed in all three exposure groups, two additional combinations with significant differences observed in only the control group, 1 additional observed in the mint-exposed group, and two additional observed in the hexanal-exposed group (Kruskal–Wallis test with

Tukey’s multiple comparisons). Full details about statistical comparisons available in Table 2.

Early odorant exposure increases the number and reliability of excitatory responses

The proportion of excitatory responses increases following prenatal and early postnatal odorant exposure (Fig. 5A–D; Table 3; ANOVA with Tukey’s multiple comparisons test; $p < 0.0001$; medians: control, 0.88; mint, 0.9235; hexanal, 0.9377; significant Tukey’s tests for control vs mint and control vs hexanal). The mint-exposed and hexanal-exposed groups both had a significantly larger proportion of excitatory responses than the control group.

Table 1. Comparisons of excitatory response amplitude, 1% odorant concentration

Comparison	Test	p value	Significant	Median control (C) C; N = 225	Median mint (M) M; N = 268	Median hexanal (H) H; N = 369
Peak ΔF/F amplitude						
IAA 10%; all groups	Kruskal--Wallis	<0.0001	Yes	0.216	0.3547	0.3697
IAA 10%; C vs M	Dunn's	<0.0001	Yes	0.216	0.3547	
IAA 10%; C vs H	Dunn's	<0.0001	Yes	0.216		0.3697
IAA 10%; H vs M	Dunn's	0.0967	No		0.3547	0.3697
IAA 1%; all groups	Kruskal--Wallis	<0.0001	Yes	0.2018	0.2389	0.3306
IAA 1%; C vs M	Dunn's	0.0745	No	0.2018	0.2389	
IAA 1%; C vs H	Dunn's	<0.0001	Yes	0.2018		0.3306
IAA 1%; H vs M	Dunn's	0.0001	Yes		0.2389	0.3306
AP all groups	Kruskal--Wallis	<0.0001	Yes	0.1081	0.1837	0.2822
AP; C vs M	Dunn's	<0.0001	Yes	0.1081	0.1837	
AP; C vs H	Dunn's	<0.0001	Yes	0.1081		0.2822
AP; H vs M	Dunn's	<0.0001	Yes		0.1837	0.2822
MS 10%; all groups	Kruskal--Wallis	<0.0001	Yes	0.1026	0.1961	0.2566
MS 10%; C vs M	Dunn's	<0.0001	Yes	0.1026	0.1961	
MS 10%; C vs H	Dunn's	<0.0001	Yes	0.1026		0.2566
MS 10%; H vs M	Dunn's	0.0143	Yes		0.1961	0.2566
IAA 5%; all groups	Kruskal--Wallis	<0.0001	Yes	0.1404	0.2386	0.2973
IAA 5%; C vs M	Dunn's	<0.0001	Yes	0.1404	0.2386	
IAA 5%; C vs H	Dunn's	<0.0001	Yes	0.1404		0.2973
IAA 5%; H vs M	Dunn's	0.0023	Yes		0.2386	0.2973
Hexanal 1%; all groups	Kruskal--Wallis	<0.0001	Yes	0.2409	0.3421	0.4718
Hexanal 1%; C vs M	Dunn's	<0.0001	Yes	0.2409	0.3421	
Hexanal 1%; C vs H	Dunn's	<0.0001	Yes	0.2409		0.4718
Hexanal 1%; H vs M	Dunn's	0.0001	Yes		0.3421	0.4718
EB all groups	Kruskal--Wallis	<0.0001	Yes	0.2262	0.3254	0.3204
EB; C vs M	Dunn's	<0.0001	Yes	0.2262	0.3254	
EB; C vs H	Dunn's	<0.0001	Yes	0.2262		0.3204
EB; H vs M	Dunn's	0.6847	No		0.3254	0.3204
MS 1%; all groups	Kruskal--Wallis	<0.0001	Yes	0.1167	0.1641	0.1933
MS 1%; C vs M	Dunn's	0.0028	Yes	0.1167	0.1641	
MS 1%; C vs H	Dunn's	<0.0001	Yes	0.1167		0.1933
MS 1%; H vs M	Dunn's	0.0026	Yes		0.1641	0.1933
PA all groups	Kruskal--Wallis	<0.0001	Yes	0.0733	0.2004	0.2511
PA; C vs M	Dunn's	<0.0001	Yes	0.0733	0.2004	
PA; C vs H	Dunn's	<0.0001	Yes	0.0733		0.2511
PA; H vs M	Dunn's	0.0244	Yes		0.2004	0.2511
MS 5%; all groups	Kruskal--Wallis	<0.0001	Yes	0.0777	0.1796	0.2503
MS 5%; C vs M	Dunn's	<0.0001	Yes	0.0777	0.1796	
MS 5%; C vs H	Dunn's	<0.0001	Yes	0.0777		0.2503
MS 5%; H vs M	Dunn's	<0.0001	Yes		0.1796	0.2503
Hexanone; all groups	Kruskal--Wallis	<0.0001	Yes	0.3185	0.4161	0.421
Hexanone; C vs M	Dunn's	<0.0001	Yes	0.3185	0.4161	
Hexanone; C vs H	Dunn's	<0.0001	Yes	0.3185		0.421
Hexanone; H vs M	Dunn's	0.8052	No		0.4161	0.421
Hexanal 10%; all groups	Kruskal--Wallis	<0.0001	Yes	0.2438	0.3768	0.3778
Hexanal 10%; C vs M	Dunn's	<0.0001	Yes	0.2438	0.3768	
Hexanal 10%; C vs H	Dunn's	<0.0001	Yes	0.2438		0.3778
Hexanal 10%; H vs M	Dunn's	0.9975	No		0.3768	0.3778
THA all groups	Kruskal--Wallis	<0.0001	Yes	0.1171	0.2183	0.2415
THA; C vs M	Dunn's	<0.0001	Yes	0.1171	0.2183	
THA; C vs H	Dunn's	<0.0001	Yes	0.1171		0.2415
ThA; H vs M	Dunn's	0.2795	No		0.2183	0.2415
Hexanal 5%; all groups	Kruskal--Wallis	<0.0001	Yes	0.2758	0.3534	0.5212
Hexanal 5%; C vs M	Dunn's	0.0001	Yes	0.2758	0.3534	
Hexanal 5%; C vs H	Dunn's	<0.0001	Yes	0.2758		0.5212
Hexanal 5%; H vs M	Dunn's	<0.0001	Yes		0.3534	0.5212

Statistical test results of excitatory response amplitudes of cells from control, mint-, and hexanal-exposed groups in response to odorants at 1% concentration. Data shown in Figure 3.

Table 2. Comparisons of excitatory response amplitude, multiple concentrations

Comparison	Test	<i>p</i> value	Significant	Median control (C) C; N = 225	Median mint (M) M; N = 268	Median hexanal (H) H; N = 369
Peak ΔF/F amplitude						
Control group						
MS; all conc.	Kruskal--Wallis	0.0586	No	0.1167	0.0777	0.1026
MS; 1% vs 5%	Dunn's	0.0508	Yes	0.1167	0.0777	
MS; 1% vs 10%	Dunn's	0.4478	No	0.1167		0.1026
MS; 5% vs 10%	Dunn's	0.4695	No		0.0777	0.1026
IAA; all conc.	Kruskal--Wallis	<0.0001	Yes	0.2018	0.1404	0.216
IAA; 1% vs 5%	Dunn's	0.0028	Yes	0.2018	0.1404	
IAA; 1% vs 10%	Dunn's	0.768	No	0.2018		0.216
IAA; 5% vs 10%	Dunn's	0.0001	Yes		0.1404	0.216
Hexanal; all conc.	Kruskal--Wallis	0.0214	Yes	0.2409	0.2758	0.2438
Hexanal; 1% vs 5%	Dunn's	0.1657	No	0.2409	0.2758	
Hexanal; 1% vs 10%	Dunn's	0.8025	No	0.2409		0.2438
Hexanal; 5% vs 10%	Dunn's	0.0206	Yes		0.2758	0.2438
Mint group						
MS; all conc.	Kruskal--Wallis	0.206	No	0.1642	0.1796	0.1961
MS; 1% vs 5%	Dunn's	0.0194	Yes	0.1642	0.1796	
MS; 1% vs 10%	Dunn's	0.1692	No	0.1642		0.1961
MS; 5% vs 10%	Dunn's	0.7856	No		0.1796	0.1961
IAA; all conc.	Kruskal--Wallis	<0.0001	Yes	0.2389	0.2386	0.3547
IAA; 1% vs 5%	Dunn's	0.9991	No	0.2389	0.2386	
IAA; 1% vs 10%	Dunn's	0.0001	Yes	0.2389		0.3547
IAA; 5% vs 10%	Dunn's	0.0001	Yes		0.2386	0.3547
Hexanal; all conc.	Kruskal--Wallis	0.3426	No	0.3421	0.3534	0.3768
Hexanal; 1% vs 5%	Dunn's	0.7615	No	0.3421	0.3534	
Hexanal; 1% vs 10%	Dunn's	0.3775	No	0.3421		0.3768
Hexanal; 5% vs 10%	Dunn's	0.9178	No		0.3534	0.3768
Hexanal group						
MS; all conc.	Kruskal--Wallis	0.0184	Yes	0.1933	0.2503	0.2566
MS; 1% vs 5%	Dunn's	0.0146	Yes	0.1933	0.2503	
MS; 1% vs 10%	Dunn's	0.2718	No	0.1933		0.2566
MS; 5% vs 10%	Dunn's	0.5645	No		0.2503	0.2566
IAA; all conc.	Kruskal--Wallis	0.0433	Yes	0.3306	0.2973	0.3697
IAA; 1% vs 5%	Dunn's	0.8326	No	0.3306	0.2973	
IAA; 1% vs 10%	Dunn's	0.2488	No	0.3306		0.3697
IAA; 5% vs 10%	Dunn's	0.0425	Yes		0.2973	0.3697
Hexanal; all conc.	Kruskal--Wallis	<0.0001	Yes	0.4718	0.5212	0.3778
Hexanal; 1% vs 5%	Dunn's	0.3105	No	0.4718	0.5212	
Hexanal; 1% vs 10%	Dunn's	0.0137	Yes	0.4718		0.3778
Hexanal; 5% vs 10%	Dunn's	<0.0001	Yes		0.5212	0.3778

Statistical test results of excitatory response amplitudes of cells from control, mint-, and hexanal-exposed groups in response to odorants at 1%, 5%, and 10% concentration. Data shown in Figure 4.

In addition, MCs of odor-exposed animals exhibited excitatory responses to more odorants than MCs of control animals (Fig. 5E; ANOVA with Tukey's multiple comparisons test; $p < 0.0001$; medians: control, 13; mint, 14; hexanal, 14; significant Tukey's tests for control vs mint and control vs hexanal). These measures indicate that odor exposure increases excitatory MC odor-evoked responses, both in number and in number of activating odors.

Excitatory responses to certain odorants were also more reliable following odor-exposure, as measured by the proportion of successful trial presentations of each odorant (Fig. 6A-D; Table 4; data pooled across odorants; Kruskal--Wallis test with Dunn's multiple comparisons test; $p < 0.0001$; medians: 1 for each group). As detailed in the Methods section, an excitatory response is defined as a peak $\Delta F/F$ at least 3 SD above average fluorescence

intensity before stimulus onset. This increase in the reliability of MCs between control and odor-exposed groups (0.75 median control to 1 for each odor-exposed group) is significant for the following odorants: MS 1% (Kruskal--Wallis test; $p = 0.0139$), MS 5% ($p < 0.0001$), MS 10% ($p < 0.0001$), THA ($p < 0.0001$), AP ($p < 0.0001$), and PA ($p < 0.0001$; Fig. 6A; additional descriptive statistics available in Table 4). The odorants for which reliability increased following odorant exposure are the ones within the panel that elicited relatively weaker responses, as measured by comparisons of their median evoked peak $\Delta F/F$ (Figs. 3, 4). Median success values were not different between groups for other odorants, although comparisons of distributions differed – these values are described in Table 4. These data together show that early odorant exposure increases the proportion and reliability of excitatory responses in a manner not dependent on the iden-

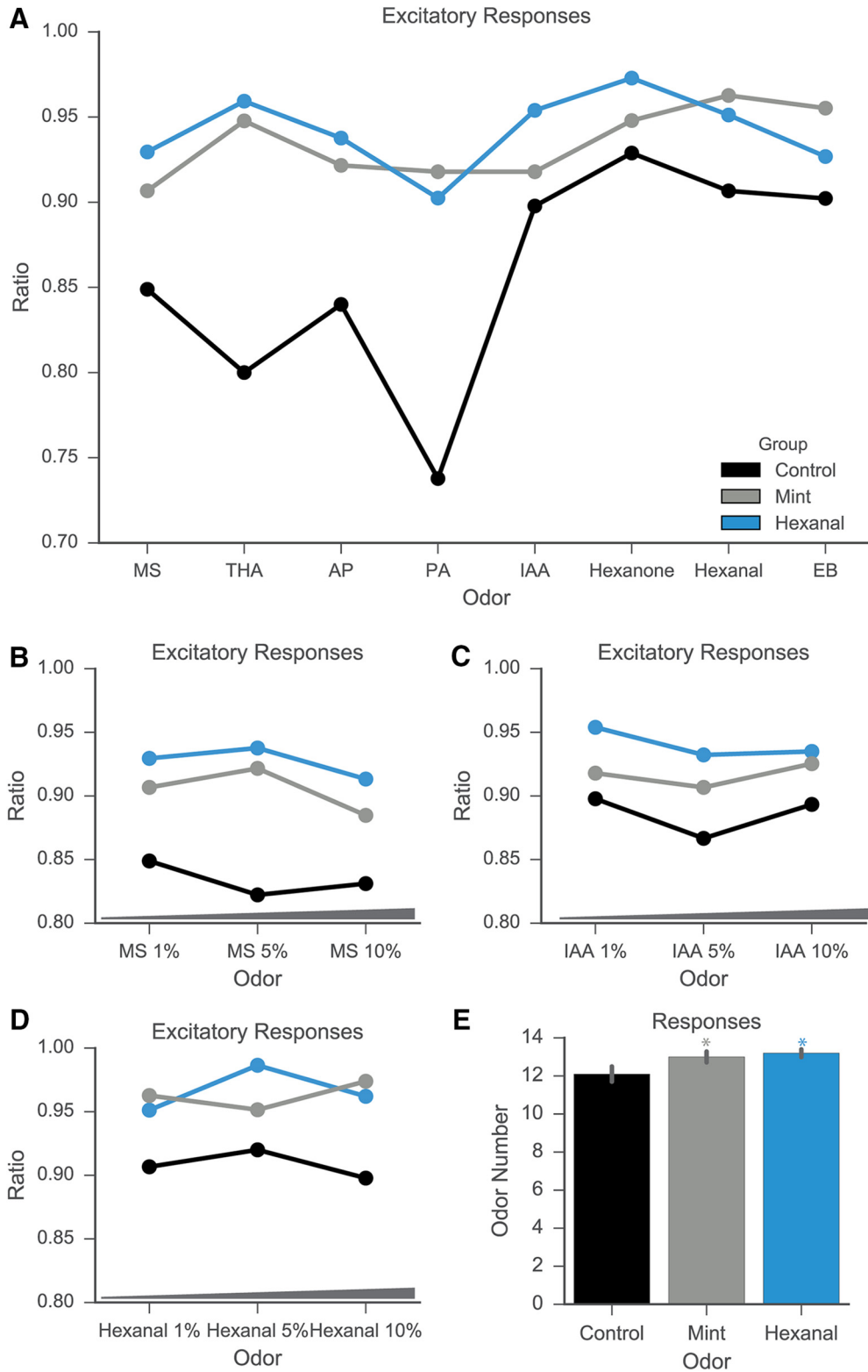


Figure 5. Odor exposure increases number of excitatory MC responses. **A--D**, Ratio of above-threshold excitatory responses to all odor presentation trials across odorants. Odor-exposure groups had significantly higher ratio of excitatory responses as compared to control groups for odors at 1% concentration (**A**) and multiple concentrations (**B--D**). **E**, MCs in all groups responded to a high number of odorants (median number of odorants: control, 13; mint exposed, 14; hexanal exposed, 14). Gray asterisk, statistically significant difference between mint and control groups; blue asterisk, statistically significant difference between hexanal and control groups.

Table 3. Proportion of excitatory responses

Comparison	Test	<i>p</i> value	Significant	Median control (C) C; N = 14	Median mint (M) M; N = 14	Median hexanal (H) H; N = 14
Ratio of excitatory responses						
All odors	ANOVA	<i>p</i> < 0.0001	Yes	0.88	0.9235	0.9377
All odors; C vs M	Tukey's	<i>p</i> < 0.0001	Yes	0.88	0.9235	
All odors; C vs H	Tukey's	<i>p</i> < 0.0001	Yes	0.88		0.9377
All odors; H vs M	Tukey's	<i>p</i> = 0.1313	No		0.9235	0.9377
Ratio of activating odorants						
All groups	Kruskal--Wallis	<i>p</i> < 0.0001	Yes	13	14	14
C vs M	Tukey's	<i>p</i> < 0.0001	Yes	13	14	
C vs H	Tukey's	<i>p</i> < 0.0001	Yes	13		14
H vs M	Tukey's	<i>p</i> > 0.9999	No		14	14

Comparisons of proportion of excitatory odor-evoked responses from control, mint-, and hexanal-exposed groups. Data shown in Figure 5.

tity of odorant used for exposure, much like the observed odor-nonspecific increases in excitatory response amplitude. Rather, the reliability of response increases in an odor-specific manner relative to the initial amplitude of odor-evoked response.

Odorant exposure changes odor tuning

Given the changes in excitatory response amplitude, number, and reliability, we next investigated whether chronic early odorant exposure also changes the odor tuning curve of cells from each exposure group. Because of the high dimensionality of odorant stimuli, we used a metric of odorant response ranking to construct a tuning curve based on the stimuli in our odor panel that can then be used to compare relative responses across exposure groups. For each cell, we assigned ranks to each odorant based on the average odor-evoked response amplitude. The odorant that evoked the highest response from the cell was assigned a rank of 14, while the odorant that evoked the lowest response from the cell was assigned a rank of 1. A nonresponse to an odorant was scored as 0 amplitude and ranked accordingly, depending on if there were inhibitory and excitatory responses within the same cell. If the cell only exhibited excitatory responses, then the nonresponse to an odorant was given the rank of 1. If two or more odorants elicited the same amplitude of odor-evoked response, such as a nonresponse, those odorants were given the same ranking. There were significant differences in the rank of specific odorants between control, mint-exposed, and hexanal-exposed groups (Fig. 7). Rather than graphically denote odorants that were different, we have listed these in table form in Figure 7E. For clarity, results of statistical comparisons and descriptive statistics are listed in Table 5. The control group (*n* = 225 cells) differed from the mint-exposed group (*n* = 269 cells) on six out of 14 possible odorant/concentration combinations (Kruskal--Wallis test followed by Dunn's multiple comparisons test). The control group differed from the hexanal-exposed group (*n* = 369 cells) on 10 out of 14 odorant/concentration combinations. Mint-exposed and hexanal-exposed groups differed on eight of 14 odorant/concentration combinations. These data show that food-based prenatal and postnatal odorant exposure does change MC tuning curves, as demonstrated by

comparisons of individual odor ranks. There are also odor-specific differences between the mint-exposed and hexanal-exposed groups, suggesting that conditioning odorant identity could impact resultant changes in odorant response ranking.

MCs exhibit habituation following repeated acute odor trials

Previous work has demonstrated that repeated presentation of odors results in a decrease in amplitude of odor-evoked responses (Chaudhury et al., 2010; Ogg et al., 2015). Given that our odor exposure paradigm did not demonstrate this effect, we examined short term habituation in a subset of animals. In three animals, one from each group, we observed acute habituation of MC response. Each of these imaging sessions took place following acquisition of MC odor-evoked responses using the complete panel of odor stimuli. Using a protocol described by Chaudhury et al. (2010), we imaged hexanal-evoked activity before stimulus, presented repeated blocks of short presentations of hexanal, and imaged hexanal-evoked activity 5 and 30 min after repeated hexanal presentation (Fig. 8A). We found prolonged decreases in odor-evoked responses 5 min and 30 min after the stimulus presentation (*n* = 56 cells) Friedman test with Tukey's multiple comparisons test; Median decrease in amplitude as percentage of initial "Pre" response: 37% for 5 min after stimulus and 35% for 30 min after stimulus; Pre versus 5 min: adjusted *p* < 0.0001; Pre versus 30 min: adjusted *p* < 0.0001; 5 vs 30 min: adjusted *p* > 0.9999 (Fig. 8B). These cells demonstrate acute habituation following repeated stimulation similar to that observed in previous studies.

Discussion

Our data show that prenatal and early postnatal food-based odor exposure increases the amplitude, number, and reliability of excitatory MC responses *in vivo*, as measured by 2-photon calcium imaging. We observed subtle changes to MC tuning curves between exposure groups, but we did not observe any changes in MC response that were specific to the conditioned odor. The mechanisms of these widespread changes are unclear and contrast with previous work using long-term postna-

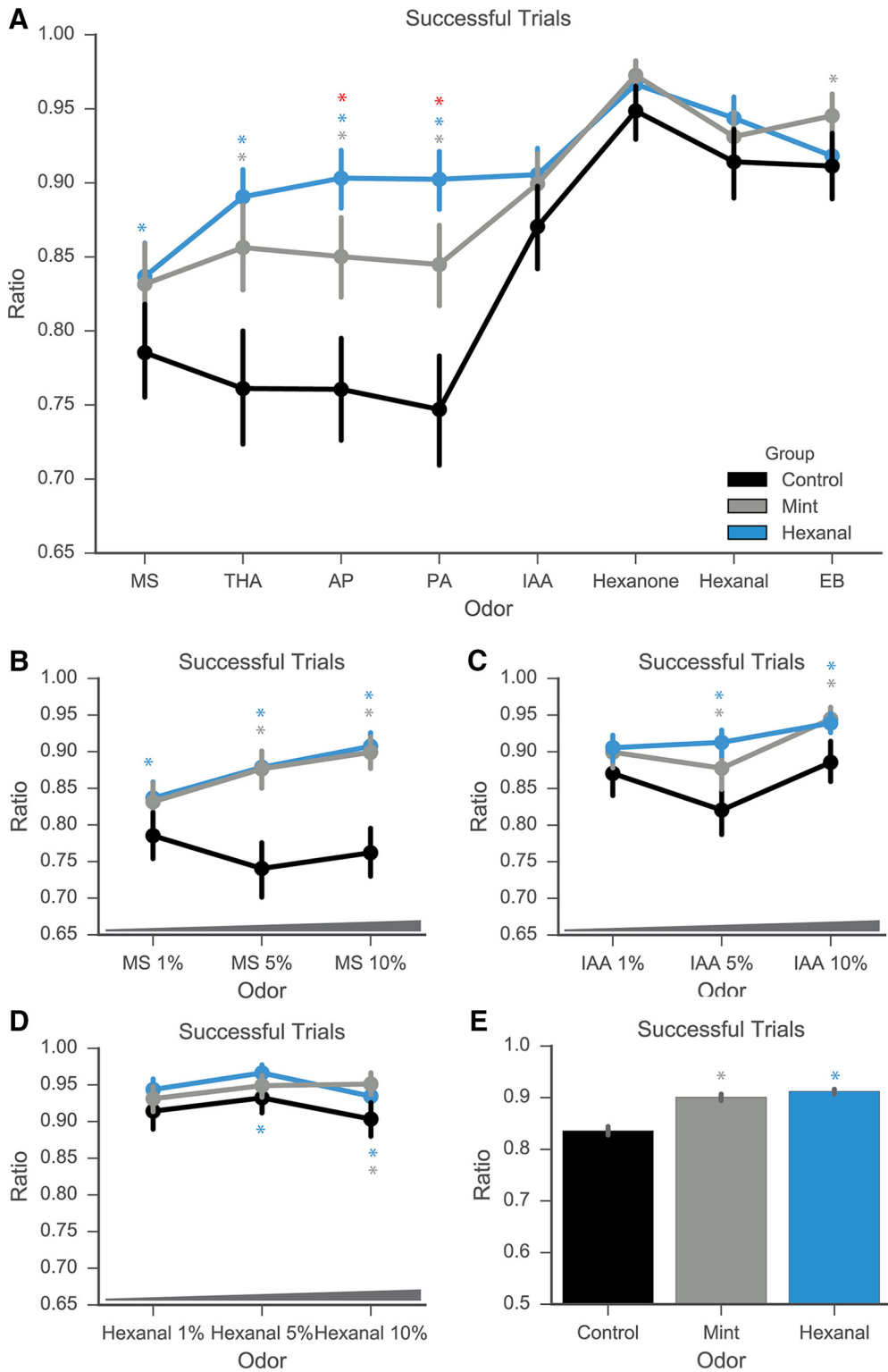


Figure 6. Odor exposure increases the rate of successful responses to odorant presentation. **A--D**, Ratio of successful trials above threshold (3 SD above baseline) to total trials of odorant presentation increases in odor-exposed groups across specific odorants at 1% concentration (**A**) and multiple concentrations of MS (**B**) and IAA (**C**), but not hexanal (**D**). **E**, Summed across all trials, odor-exposed groups had higher overall ratio of successful trials. Gray asterisk, statistically significant difference between mint and control groups; blue asterisk, statistically significant difference between hexanal and control; red asterisk, statistically significant difference between hexanal and mint.

Table 4. Reliability of excitatory responses

Comparison	Test	<i>p</i> value	Median control (C) C; N = 225	Median mint (M) M; N = 268	Median hexanal (H) H; N = 369
Ratio of successful trials					
All odors combined; all groups	Kruskal--Wallis	<0.0001	1	1	1
All odors combined; C vs M	Dunn's	<0.0001	1	1	
All odors combined; C vs H	Dunn's	<0.0001	1		1
All odors combined; H vs M	Dunn's	0.0836		1	1
AP all groups	Kruskal--Wallis	<0.0001	0.75	1	1
AP; C vs M	Dunn's	<0.0001	0.75	1	
AP; C vs H	Dunn's	<0.0001	0.75		1
AP; H vs M	Dunn's	0.0029		1	1
EB all groups	Kruskal--Wallis	0.0181	1	1	1
EB; C vs M	Dunn's	0.0151	1	1	
EB; C vs H	Dunn's	0.5476	1		1
EB; H vs M	Dunn's	0.2351		1	1
Hexanal 1%; all groups	Kruskal--Wallis	0.0596	1	1	1
Hexanal 1%; C vs M	Dunn's	0.7821	1	1	
Hexanal 1%; C vs H	Dunn's	0.0562	1		1
Hexanal 1%; H vs M	Dunn's	0.6464		1	1
Hexanone; all groups	Kruskal--Wallis	0.1165	1	1	1
Hexanone; C vs M	Dunn's	0.1277	1	1	
Hexanone; C vs H	Dunn's	0.3668	1		1
Hexanone; H vs M	Dunn's	>0.9999		1	1
IAA 1% all groups	Kruskal--Wallis	0.0902	1	1	1
IAA 1%; C vs M	Dunn's	0.4431	1		1
IAA 1%; C vs H	Dunn's	0.0867	1	1	
IAA 1%; H vs M	Dunn's	>0.9999		1	1
MS 1%	Kruskal--Wallis	0.0139	0.75	1	1
all groups					
MS 1%; C vs M	Dunn's	0.0621	0.75	1	
MS 1%; C vs H	Dunn's	0.0144	0.75		1
MS 1%; H vs M	Dunn's	>0.9999		1	1
PA all groups	Kruskal--Wallis	<0.0001	0.75	1	1
PA; C vs M	Dunn's	<0.0001	0.75	1	
PA; C vs H	Dunn's	<0.0001	0.75		1
PA; H vs M	Dunn's	0.0019		1	1
THA all groups	Kruskal--Wallis	<0.0001	0.75	1	1
THA; C vs M	Dunn's	<0.0001	0.75	1	
THA; C vs H	Dunn's	<0.0001	0.75		1
THA; H vs M	Dunn's	0.9588		1	1
MS 5%; all groups	Kruskal--Wallis	<0.0001	0.75	1	1
MS 5%; C vs M	Dunn's	<0.0001	0.75	1	
MS 5%; C vs H	Dunn's	<0.0001	0.75		1
MS 5%; H vs M	Dunn's	>0.9999		1	1
MS 10%; all groups	Kruskal--Wallis	<0.0001	0.75	1	1
MS 10%; C vs M	Dunn's	<0.0001	0.75	1	
MS 10%; C vs H	Dunn's	<0.0001	0.75		1
MS 10%; H vs M	Dunn's	>0.9999		1	1
Hexanal 5%; all groups	Kruskal--Wallis	0.0016	1	1	1
Hexanal 5%; C vs M	Dunn's	0.4815	1	1	
Hexanal 5%; C vs H	Dunn's	0.0014	1		1
Hexanal 5%; H vs M	Dunn's	0.1031		1	1
Hexanal 10%; all groups	Kruskal--Wallis	0.0001	1	1	1
Hexanal 10%; C vs M	Dunn's	0.0001	1	1	
Hexanal 10%; C vs H	Dunn's	0.0029	1		1
Hexanal 10%; H vs M	Dunn's	0.81		1	1
IAA 5%; all groups	Kruskal--Wallis	<0.0001	1	1	1
IAA 5%; C vs M	Dunn's	0.0026	1	1	
IAA 5%; C vs H	Dunn's	<0.0001	1		1
IAA 5%; H vs M	Dunn's	0.4956		1	1
IAA 10%; all groups	Kruskal--Wallis	0.0007	1	1	1
IAA 10%; C vs M	Dunn's	0.0007	1	1	
IAA 10%; C vs H	Dunn's	0.01	1		1
IAA 10%; H vs M	Dunn's	0.8374		1	1

Comparisons of excitatory response reliability from control, mint-, and hexanal-exposed groups. Data shown in Figure 6.

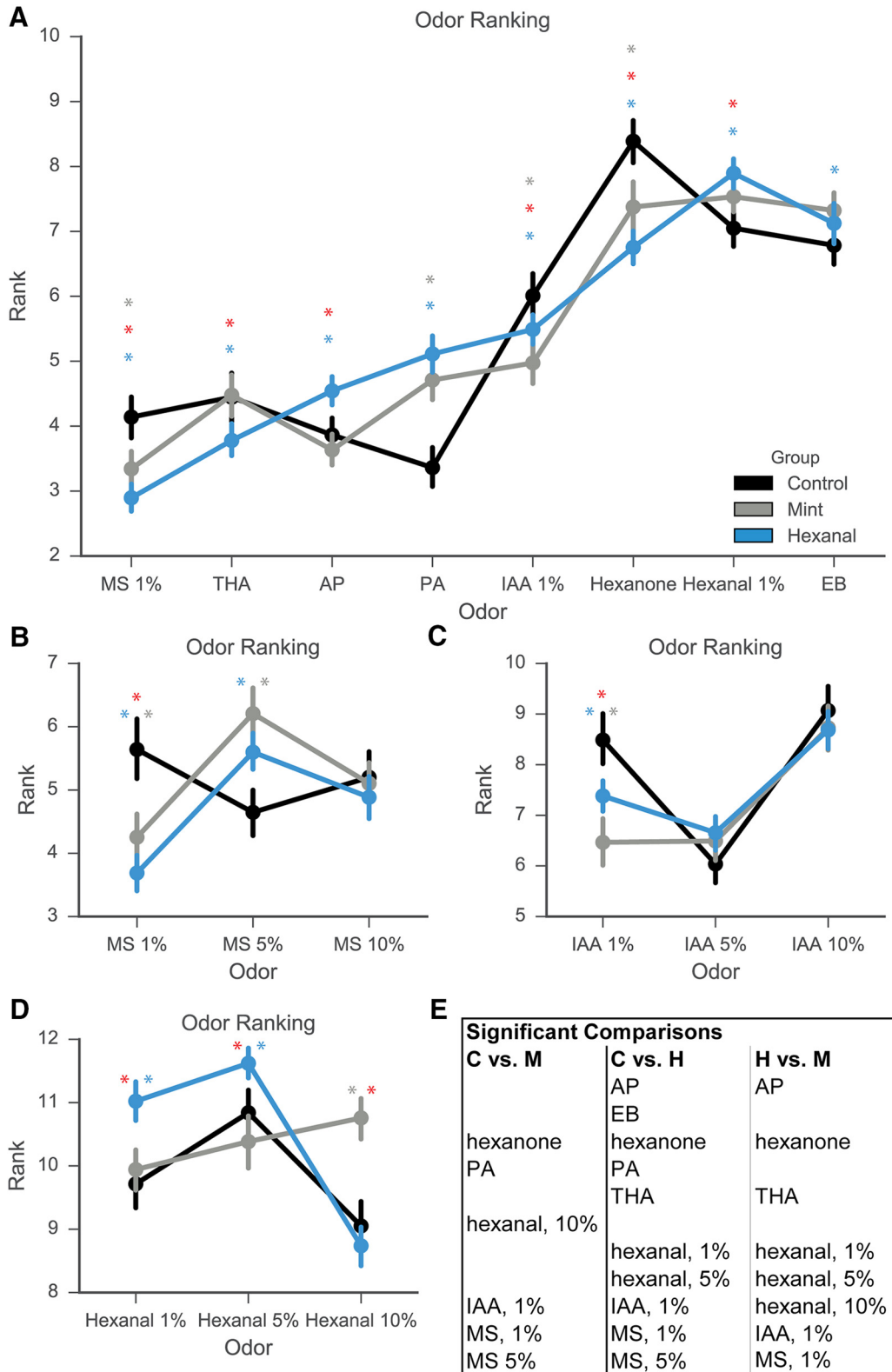


Figure 7. Odor ranking changes for specific odorants following odor exposure. **A**, Response rank for each odor calculated on a cell-by-cell basis. Odorants at 1% concentration displayed. **B--D**, Response rank for multiple concentrations of MS (**B**), IAA (**C**), and hexanal (**D**). **E**, Table of significant differences in odorant ranks for each group comparison. Gray asterisk, statistically significant difference between mint and control groups; blue asterisk, statistically significant difference between hexanal and control; red asterisk, statistically significant difference between hexanal and mint.

Table 5. Comparison of odor ranks

Comparison	Test	<i>p</i> value	Significant	Median control (C) C; N = 225	Median mint (M) M; N = 268	Median hexanal (H) H; N = 369
Response rank						
AP all groups	Kruskal--Wallis	<0.0001	Yes	5	4	6
AP; C vs M	Dunn's	0.417	No	5	4	
AP; C vs H	Dunn's	0.0002	Yes	5		6
AP; H vs M	Dunn's	<0.0001	Yes		4	6
EB all groups	Kruskal--Wallis	0.0176	Yes	10	10	11
EB; C vs M	Dunn's	0.2086	No	10	10	
EB; C vs H	Dunn's	0.0138	Yes	10		11
EB; H vs M	Dunn's	>0.9999	No		10	11
Hexanal 1%; all groups	Kruskal--Wallis	<0.0001	Yes	10	10	12
Hexanal 1%; C vs M	Dunn's	>0.9999	No	10	10	
Hexanal 1%; C vs H	Dunn's	<0.0001	Yes	10		12
Hexanal 1%; H vs M	Dunn's	<0.0001	Yes		10	12
Hexanone; all groups	Kruskal--Wallis	<0.0001	Yes	13	11	11
Hexanone; C vs M	Dunn's	<0.0001	Yes	13	11	
Hexanone; C vs H	Dunn's	<0.0001	Yes	13		11
Hexanone; H vs M	Dunn's	0.0001	Yes		11	11
IAA 1% all groups	Kruskal--Wallis	<0.0001	Yes	9	6	7
IAA 1%; C vs M	Dunn's	<0.0001	Yes	9	6	
IAA 1%; C vs H	Dunn's	0.0004	Yes	9		7
IAA 1%; H vs M	Dunn's	0.0065	Yes		6	7
MS 1% all groups	Kruskal--Wallis	<0.0001	Yes	5	3	3
MS 1%; C vs M	Dunn's	<0.0001	Yes	5	3	
MS 1%; C vs H	Dunn's	<0.0001	Yes	5		3
MS 1%; H vs M	Dunn's	0.0391	Yes		3	3
PA all groups	Kruskal--Wallis	<0.0001	Yes	4	5	7
PA; C vs M	Dunn's	<0.0001	Yes	4	5	
PA; C vs H	Dunn's	<0.0001	Yes	4		7
PA; H vs M	Dunn's	0.0908	No		5	7
THA all groups	Kruskal--Wallis	0.0028	Yes	5	5	4
THA; C vs M	Dunn's	>0.9999	No	5	5	
THA; C vs H	Dunn's	0.0234	Yes	5		4
THA; H vs M	Dunn's	0.0071	Yes		5	4
MS 5%; all groups	Kruskal--Wallis	<0.0001	Yes	4	6	5
MS 5%; C vs M	Dunn's	<0.0001	Yes	4	6	
MS 5%; C vs H	Dunn's	0.0001	Yes	4		5
MS 5%; H vs M	Dunn's	0.4328	No		6	5
MS 10%; all groups	Kruskal--Wallis	0.0731	No	5	5	4
MS 10%; C vs M	Dunn's	>0.9999	No	5	5	
MS 10%; C vs H	Dunn's	0.1503	No	5		4
MS 10%; H vs M	Dunn's	0.1864	No		5	4
IAA 5%; all groups	Kruskal--Wallis	0.1561	No	7	7	6
IAA 5%; C vs M	Dunn's	0.3965	No	7	7	
IAA 5%; C vs H	Dunn's	0.1912	No	7		6
IAA 5%; H vs M	Dunn's	>0.9999	No		7	6
IAA 10%; all groups	Kruskal--Wallis	0.3245	No	10	9	9
IAA 10%; C vs M	Dunn's	0.6739	No	10	9	
IAA 10%; C vs H	Dunn's	0.4643	No	10		9
IAA 10%; H vs M	Dunn's	>0.9999	No		9	9
Hexanal 5%; all groups	Kruskal--Wallis	<0.0001	Yes	12	12	12
Hexanal 5%; C vs M	Dunn's	>0.9999	No	12	12	
Hexanal 5%; C vs H	Dunn's	0.0012	Yes	12		12
Hexanal 5%; H vs M	Dunn's	<0.0001	Yes		12	12
Hexanal 10%; all groups	Kruskal--Wallis	<0.0001	Yes	9	12	9
Hexanal 10%; C vs M	Dunn's	<0.0001	Yes	9	12	
Hexanal 10%; C vs H	Dunn's	0.7317	No	9		9
Hexanal 10%; H vs M	Dunn's	<0.0001	Yes		12	9

Statistical test results of odor ranks between control, mint-, and hexanal-exposed groups. Data shown in Figure 7.

tal odor enrichment and acute habituation (Wilson et al., 1985, 1987; Kato et al., 2012). Key differences between our experiments and this previous work include the tim-

ing, duration, and method of odor exposure, suggesting that exposure context can modulate the effects of sensory experience on the OB. It was previously observed

Table 6. Statistical values

Statistical values	Data structure	Type of test	Power
For all multiple comparisons tests, reported p is adjusted p value			
Figure 2			
Distribution of responses; all groups; $p = 6.65E-199$	Non-normal	Kruskal--Wallis	KW statistic: 912.64
Distribution of responses; C vs M; p approximates 0	Non-normal	Tukey's	95% CI: -1.8211 to -1.4263
Distribution of responses; C vs H; p approximates 0	Non-normal	Tukey's	95% CI: -2.5607 to -2.1911
Distribution of responses; H vs M; p approximates 0	Non-normal	Tukey's	95% CI: -0.9273 to -0.577
Figure 3			
Excitation amplitude; IAA 10%; all groups; $p < 0.0001$	Non-normal	Kruskal--Wallis	
Excitation amplitude; IAA 10%; C (med: 0.216) vs M (0.3547); $p < 0.0001$	Non-normal	Tukey's	
Excitation amplitude; IAA 10%; C vs H (med: 0.3697); $p < 0.0001$	Non-normal	Tukey's	
Excitation amplitude; IAA 10%; H vs M; $p = 0.0842$	Non-normal	Tukey's	
Excitation amplitude; IAA 1%; all groups; $p < 0.0001$	Non-normal	Kruskal--Wallis	
Excitation amplitude; IAA 1%; C (med: 0.2018) vs M (0.2389); $p = 0.0656$	Non-normal	Tukey's	
Excitation amplitude; IAA 1%; C vs H (0.3306); $p < 0.0001$	Non-normal	Tukey's	
Excitation amplitude; IAA 1%; H vs M; $p = 0.0001$	Non-normal	Tukey's	
Excitation amplitude; AP; all groups; $p < 0.0001$	Non-normal	Kruskal--Wallis	
Excitation amplitude; AP; C (med: 0.1081) vs M (0.1837); $p < 0.0001$	Non-normal	Tukey's	
Excitation amplitude; AP; C vs H (med: 0.2822); $p < 0.0001$	Non-normal	Tukey's	
Excitation amplitude; AP; H vs M; $p < 0.0001$	Non-normal	Tukey's	
Excitation amplitude; MS 10%; all groups; $p < 0.0001$	Non-normal	Kruskal--Wallis	
Excitation amplitude; MS 10%; C (med: 0.1026) vs M (0.1961); $p < 0.0001$	Non-normal	Tukey's	
Excitation amplitude; MS 10%; C vs H (med: 0.2566); $p < 0.0001$	Non-normal	Tukey's	
Excitation amplitude; MS 10%; H vs M; $p = 0.0022$	Non-normal	Tukey's	
Excitation amplitude; IAA 5%; all groups; $p < 0.0001$	Non-normal	Kruskal--Wallis	
Excitation amplitude; IAA 5%; C (med: 0.1404) vs M (0.2386); $p < 0.0001$	Non-normal	Tukey's	
Excitation amplitude; IAA 5%; C vs H (med: 0.2973); $p < 0.0001$	Non-normal	Tukey's	
Excitation amplitude; IAA 5%; H vs M; $p = 0.0022$	Non-normal	Tukey's	
Excitation amplitude; Hexanal 1%; all groups; $p < 0.0001$	Non-normal	Kruskal--Wallis	
Excitation amplitude; Hexanal 1%; C (med: 0.2409) vs M (0.3421); $p < 0.0001$	Non-normal	Tukey's	
Excitation amplitude; Hexanal 1%; C vs H (med: 0.4718); $p < 0.0001$	Non-normal	Tukey's	
Excitation amplitude; Hexanal 1%; H vs M; $p = 0.0001$	Non-normal	Tukey's	
Excitation amplitude; EB; all groups; $p < 0.0001$	Non-normal	Kruskal--Wallis	
Excitation amplitude; EB; C (med: 0.2262) vs M (0.3254); $p < 0.0001$	Non-normal	Tukey's	
Excitation amplitude; EB; C vs H (0.3204); $p < 0.0001$	Non-normal	Tukey's	
Excitation amplitude; EB; H vs M; $p = 0.5796$	Non-normal	Tukey's	
Excitation amplitude; MS 1%; all groups; $p < 0.0001$	Non-normal	Kruskal--Wallis	
Excitation amplitude; MS 1%; C (med: 0.1167) vs M (0.1641); $p = 0.0027$	Non-normal	Tukey's	
Excitation amplitude; MS 1%; C vs H (med: 0.1933); $p < 0.0001$	Non-normal	Tukey's	
Excitation amplitude; MS 1%; H vs M; $p = 0.0224$	Non-normal	Tukey's	
Excitation amplitude; PA; all groups; $p < 0.0001$	Non-normal	Kruskal--Wallis	
Excitation amplitude; PA; C (med: 0.0733) vs M (0.2004); $p < 0.0001$	Non-normal	Tukey's	
Excitation amplitude; PA; C vs H (med: 0.2511); $p < 0.0001$	Non-normal	Tukey's	
Excitation amplitude; PA; H vs M; $p = 0.0224$	Non-normal	Tukey's	
Excitation amplitude; MS 5%; all groups; $p < 0.0001$	Non-normal	Kruskal--Wallis	
Excitation amplitude; MS 5%; C (med: 0.0777) vs M (0.1796); $p < 0.0001$	Non-normal	Tukey's	
Excitation amplitude; MS 5%; C vs H (med: 0.2503); $p < 0.0001$	Non-normal	Tukey's	
Excitation amplitude; MS 5%; H vs M; $p = 0.6994$	Non-normal	Tukey's	
Excitation amplitude; Hexanone; all groups; $p < 0.0001$	Non-normal	Kruskal--Wallis	
Excitation amplitude; Hexanone; C (med: 0.3185) vs M (0.4161); $p < 0.0001$	Non-normal	Tukey's	
Excitation amplitude; Hexanone; C vs H (med: 0.421); $p < 0.0001$	Non-normal	Tukey's	
Excitation amplitude; Hexanone; H vs M; $p = 0.6994$	Non-normal	Tukey's	
Excitation amplitude; Hexanal 10%; all groups; $p < 0.0001$	Non-normal	Kruskal--Wallis	
Excitation amplitude; Hexanal 10%; C (med: 0.2438) vs M (0.3768); $p < 0.0001$	Non-normal	Tukey's	
Excitation amplitude; Hexanal 10%; C vs H (med: 0.3778); $p < 0.0001$	Non-normal	Tukey's	
Excitation amplitude; Hexanal 10%; H vs M; $p = 0.9842$	Non-normal	Tukey's	
Excitation amplitude; THA; all groups; $p < 0.0001$	Non-normal	Kruskal--Wallis	
Excitation amplitude; THA; C (med: 0.1171) vs M (0.2183); $p < 0.0001$	Non-normal	Tukey's	
Excitation amplitude; THA; C vs H (med: 0.2415); $p < 0.0001$	Non-normal	Tukey's	
Excitation amplitude; THA; H vs M; $p = 0.2338$	Non-normal	Tukey's	
Excitation amplitude; Hexanal 5%; all groups; $p < 0.0001$	Non-normal	Kruskal--Wallis	
Excitation amplitude; Hexanal 5%; C (med: 0.2758) vs M (0.3534); $p = 0.0001$	Non-normal	Tukey's	
Excitation amplitude; Hexanal 5%; C vs H (med: 0.5212); $p < 0.0001$	Non-normal	Tukey's	
Excitation amplitude; Hexanal 5%; H vs M; $p < 0.0001$	Non-normal	Tukey's	
Figure 4, concentration comparisons			
Control group			
Excitation amplitude; MS; all conc.; $p = 0.0586$	Non-normal	Kruskal--Wallis	
Excitation amplitude; MS; 1% (med: 0.1167) vs 5% (0.0777); $p = 0.0454$	Non-normal	Tukey's	
Excitation amplitude; MS; 1% vs 10% (med: 0.1026); $p = 0.4478$	Non-normal	Tukey's	
Excitation amplitude; MS; 5% vs 10%; $p = 0.4695$	Non-normal	Tukey's	
Excitation amplitude; IAA; all conc.; $p < 0.0001$	Non-normal	Kruskal--Wallis	
Excitation amplitude; IAA; 1% (med: 0.2018) vs 5% (0.1404); $p = 0.0027$	Non-normal	Tukey's	
Excitation amplitude; IAA; 1% vs 10% (med: 0.216); $p = 0.6607$	Non-normal	Tukey's	
Excitation amplitude; IAA; 5% vs 10%; $p = 0.0001$	Non-normal	Tukey's	

(Continued)

Table 6. Continued

Statistical values	Data structure	Type of test	Power
Excitation amplitude; hexanal; all conc.; $p = 0.0214$	Non-normal	Kruskal--Wallis	
Excitation amplitude; hexanal; 1% (med: 0.2409) vs 5% (0.2758); $p = 0.1412$	Non-normal	Tukey's	
Excitation amplitude; hexanal; 1% vs 10% (med: 0.2438); $p = 0.6965$	Non-normal	Tukey's	
Excitation amplitude; hexanal; 5% vs 10%; $p = 0.0189$	Non-normal	Tukey's	
Mint group			
Excitation amplitude; MS; all conc.; $p = 0.206$	Non-normal	Kruskal--Wallis	
Excitation amplitude; MS; 1% (med: 0.1641) vs 5% (0.1796); $p = 0.0179$	Non-normal	Tukey's	
Excitation amplitude; MS; 1% vs 10% (med: 0.1961); $p = 0.1441$	Non-normal	Tukey's	
Excitation amplitude; MS; 5% vs 10%; $p = 0.6787$	Non-normal	Tukey's	
Excitation amplitude; IAA; all conc.; $p < 0.0001$	Non-normal	Kruskal--Wallis	
Excitation amplitude; IAA; 1% (med: 0.2389) vs 5% (0.2386); $p = 0.9919$	Non-normal	Tukey's	
Excitation amplitude; IAA; 1% vs 10% (med: 0.3547); $p = 0.0001$	Non-normal	Tukey's	
Excitation amplitude; IAA; 5% vs 10%; $p = 0.0001$	Non-normal	Tukey's	
Excitation amplitude; hexanal; all conc.; $p = 0.3426$	Non-normal	Kruskal--Wallis	
Excitation amplitude; hexanal; 1% (med: 0.3421) vs 5% (0.3534); $p = 0.6541$	Non-normal	Tukey's	
Excitation amplitude; hexanal; 1% vs 10% (med: 0.3768); $p = 0.3137$	Non-normal	Tukey's	
Excitation amplitude; hexanal; 5% vs 10%; $p = 0.8335$	Non-normal	Tukey's	
Hexanal group			
Excitation amplitude; MS; all conc.; $p = 0.0184$	Non-normal	Kruskal--Wallis	
Excitation amplitude; MS; 1% (med: 0.1933) vs 5% (0.2503); $p = 0.0136$	Non-normal	Tukey's	
Excitation amplitude; MS; 1% vs 10% (med: 0.2566); $p = 0.2275$	Non-normal	Tukey's	
Excitation amplitude; MS; 5% vs 10%; $p = 0.4712$	Non-normal	Tukey's	
Excitation amplitude; IAA; all conc.; $p = 0.0433$	Non-normal	Kruskal--Wallis	
Excitation amplitude; IAA; 1% (med: 0.3306) vs 5% (0.2973); $p = 0.7292$	Non-normal	Tukey's	
Excitation amplitude; IAA; 1% vs 10% (0.3697); $p = 0.2089$	Non-normal	Tukey's	
Excitation amplitude; IAA; 5% vs 10%; $p = 0.0382$	Non-normal	Tukey's	
Excitation amplitude; hexanal; all conc.; $p < 0.0001$	Non-normal	Kruskal--Wallis	
Excitation amplitude; hexanal; 1% (0.4718) vs 5% (0.5212); $p = 0.2589$	Non-normal	Tukey's	
Excitation amplitude; hexanal; 1% vs 10% (0.3778); $p = 0.0127$	Non-normal	Tukey's	
Excitation amplitude; hexanal; 5% vs 10%; $p < 0.0001$	Non-normal	Tukey's	
Figure 5			
Ratio of excitatory events; all odors; $p < 0.0001$	Normal	ANOVA	
Ratio of excitatory events; all odors: C (med: 0.88) vs M (0.9235); $p < 0.0001$	Normal	Tukey's	
Ratio of excitatory events; all odors: C vs H (med: 0.9377); $p < 0.0001$	Normal	Tukey's	
Ratio of excitatory events; all odors: H vs M; $p = 0.1313$	Normal	Tukey's	
Ratio of activating odors; all groups; $p < 0.0001$	Non-normal	Kruskal--Wallis	
Ratio of activating odors; C (med: 13) vs, M (14); $p < 0.0001$	Non-normal	Dunn's	
Ratio of activating odors; C vs H (med: 14); $p < 0.0001$	Non-normal	Dunn's	
Ratio of activating odors; H vs M; $p > 0.9999$	Non-normal	Dunn's	
Figure 6			
Successes; AP; all groups; $p < 0.0001$	Non-normal	Kruskal--Wallis	
Successes; AP; C (med: 0.75) vs M (1); $p < 0.0001$	Non-normal	Dunn's	
Successes; AP; C vs H (med: 1); $p < 0.0001$	Non-normal	Dunn's	
Successes; AP; H vs M; $p = 0.0029$	Non-normal	Dunn's	
Successes; EB; all groups; $p = 0.0181$	Non-normal	Kruskal--Wallis	
Successes; EB; C (med: 1) vs M (1); $p = 0.0151$	Non-normal	Dunn's	
Successes; EB; C vs H (1); $p = 0.5476$	Non-normal	Dunn's	
Successes; EB; H vs M; $p = 0.2351$	Non-normal	Dunn's	
Successes; Hexanal 1%; all groups; $p = 0.0596$	Non-normal	Kruskal--Wallis	
Successes; Hexanal 1%; C (med: 1) vs M (med: 1); $p = 0.7821$	Non-normal	Dunn's	
Successes; Hexanal 1%; C vs H (med: 1); $p = 0.0562$	Non-normal	Dunn's	
Successes; Hexanal 1%; H vs M; $p = 0.6464$	Non-normal	Dunn's	
Successes; Hexanone; all groups; $p = 0.1165$	Non-normal	Kruskal--Wallis	
Successes; Hexanone; C (med: 1) vs M (1); $p = 0.1277$	Non-normal	Dunn's	
Successes; Hexanone; C vs H (1); $p = 0.3668$	Non-normal	Dunn's	
Successes; Hexanone; H vs M; $p > 0.9999$	Non-normal	Dunn's	
Successes; IAA 1%; all groups; $p = 0.0902$	Non-normal	Kruskal--Wallis	
Successes; IAA 1%; C (med: 1) vs M (1); $p = 0.4431$	Non-normal	Dunn's	
Successes; IAA 1%; C vs H (1); $p = 0.0867$	Non-normal	Dunn's	
Successes; IAA 1%; H vs M; $p > 0.9999$	Non-normal	Dunn's	
Successes; MS 1%; all groups; $p = 0.0139$	Non-normal	Kruskal--Wallis	
Successes; MS 1%; C (med: 0.75) vs M (1); $p = 0.0621$	Non-normal	Dunn's	
Successes; MS 1%; C vs H (1); $p = 0.0144$	Non-normal	Dunn's	
Successes; MS 1%; H vs M; $p > 0.9999$	Non-normal	Dunn's	
Successes; PA; all groups; $p < 0.0001$	Non-normal	Kruskal--Wallis	
Successes; PA; C (med: 0.75) vs M (1); $p < 0.0001$	Non-normal	Dunn's	
Successes; PA; C vs H (1); $p < 0.0001$	Non-normal	Dunn's	
Successes; PA; H vs M; $p = 0.0019$	Non-normal	Dunn's	
Successes; THA; all groups; $p < 0.0001$	Non-normal	Kruskal--Wallis	
Successes; THA; C (med: 0.75); vs M (1); $p < 0.0001$	Non-normal	Dunn's	
Successes; THA; C vs H (1); $p < 0.0001$	Non-normal	Dunn's	
Successes; THA; H vs M; $p = 0.9588$	Non-normal	Dunn's	
Successes; MS 5%; all groups; $p < 0.0001$	Non-normal	Kruskal--Wallis	

(Continued)

Table 6. Continued

Statistical values	Data structure	Type of test	Power
Successes; MS 5%; C (med: 0.75) vs M (1); $p < 0.0001$	Non-normal	Dunn's	
Successes; MS 5%; C vs H (med: 1); $p < 0.0001$	Non-normal	Dunn's	
Successes; MS 5%; H vs M; $p > 0.9999$	Non-normal	Dunn's	
Successes; MS 10%; all groups; $p < 0.0001$	Non-normal	Kruskal--Wallis	
Successes; MS 10%; C (med: 0.75) vs M (1); $p < 0.0001$	Non-normal	Dunn's	
Successes; MS 10%; C vs H (med: 1); $p < 0.0001$	Non-normal	Dunn's	
Successes; MS 10%; H vs M; $p > 0.9999$	Non-normal	Dunn's	
Successes; Hexanal 5%; all groups; $p = 0.0016$	Non-normal	Kruskal--Wallis	
Successes; Hexanal 5%; C (med: 1) vs M (1); $p = 0.4815$	Non-normal	Dunn's	
Successes; Hexanal 5%; C vs H (med: 1); $p = 0.0014$	Non-normal	Dunn's	
Successes; Hexanal 5%; H vs M; $p = 0.1031$	Non-normal	Dunn's	
Successes; Hexanal 10%; all groups; $p = 0.0001$	Non-normal	Kruskal--Wallis	
Successes; Hexanal 10%; C (med: 1) vs M (1); $p = 0.0001$	Non-normal	Dunn's	
Successes; Hexanal 10%; C vs H (med: 1); $p = 0.0029$	Non-normal	Dunn's	
Successes; Hexanal 10%; H vs M; $p = 0.81$	Non-normal	Dunn's	
Successes; IAA 5%; all groups; $p < 0.0001$	Non-normal	Kruskal--Wallis	
Successes; IAA 5%; C (med: 1) vs M (1); $p = 0.0026$	Non-normal	Dunn's	
Successes; IAA 5%; C vs H (med: 1); $p < 0.0001$	Non-normal	Dunn's	
Successes; IAA 5%; H vs M; $p = 0.4956$	Non-normal	Dunn's	
Successes; IAA 10%; all groups; $p = 0.0007$	Non-normal	Kruskal--Wallis	
Successes; IAA 10%; C (med: 1) vs M (1); $p = 0.0007$	Non-normal	Dunn's	
Successes; IAA 10%; C vs H (med: 1); $p = 0.01$	Non-normal	Dunn's	
Successes; IAA 10%; H vs M; $p = 0.8374$	Non-normal	Dunn's	
Successes; All odors combined; all groups; $p < 0.0001$	Non-normal	Kruskal--Wallis	
Successes; All odors combined; C (med: 1) vs M (1); $p < 0.0001$	Non-normal	Dunn's	
Successes; All odors combined; C vs H (med: 1); $p < 0.0001$	Non-normal	Dunn's	
Successes; All odors combined; H vs M, $p = 0.0836$	Non-normal	Dunn's	
Figure 7			
Comparison of ranks; AP; all groups; $p < 0.0001$	Non-normal	Kruskal--Wallis	
Comparison of ranks; AP; C (med: 5) vs M (4); $p = 0.4176$	Non-normal	Dunn's	
Comparison of ranks; AP; C vs H (med: 6); $p = 0.0002$	Non-normal	Dunn's	
Comparison of ranks; AP; H vs M; $p < 0.0001$	Non-normal	Dunn's	
Comparison of ranks; EB; all groups; $p = 0.0176$	Non-normal	Kruskal--Wallis	
Comparison of ranks; EB; C (med: 10) vs M (10); $p = 0.2086$	Non-normal	Dunn's	
Comparison of ranks; EB; C vs H (med: 11); $p = 0.0138$	Non-normal	Dunn's	
Comparison of ranks; EB; H vs M; $p > 0.9999$	Non-normal	Dunn's	
Comparison of ranks; Hex 1%; all groups; $p < 0.0001$	Non-normal	Kruskal--Wallis	
Comparison of ranks; Hex 1%; C (med: 10) vs M (10); $p > 0.9999$	Non-normal	Dunn's	
Comparison of ranks; Hex 1%; C vs H (med: 12); $p < 0.0001$	Non-normal	Dunn's	
Comparison of ranks; Hex 1%; H vs M; $p < 0.0001$	Non-normal	Dunn's	
Comparison of ranks; Hexanone; all groups; $p < 0.0001$	Non-normal	Kruskal--Wallis	
Comparison of ranks; Hexanone; C (med: 13) vs M (11); $p < 0.0001$	Non-normal	Dunn's	
Comparison of ranks; Hexanone; C vs H (med: 11); $p < 0.0001$	Non-normal	Dunn's	
Comparison of ranks; Hexanone; H vs M; $p = 0.0001$	Non-normal	Dunn's	
Comparison of ranks; IAA 1%; all groups; $p < 0.0001$	Non-normal	Kruskal--Wallis	
Comparison of ranks; IAA 1%; C (med: 9) vs M (6); $p < 0.0001$	Non-normal	Dunn's	
Comparison of ranks; IAA 1%; C vs H (med: 7); $p = 0.0004$	Non-normal	Dunn's	
Comparison of ranks; IAA 1%; H vs M; $p = 0.0065$	Non-normal	Dunn's	
Comparison of ranks; MS 1%; all groups; $p < 0.0001$	Non-normal	Kruskal--Wallis	
Comparison of ranks; MS 1%; C (med: 5) vs M (3); $p < 0.0001$	Non-normal	Dunn's	
Comparison of ranks; MS 1%; C vs H (med: 3); $p < 0.0001$	Non-normal	Dunn's	
Comparison of ranks; MS 1%; H vs M; $p = 0.0391$	Non-normal	Dunn's	
Comparison of ranks; PA; all groups; $p < 0.0001$	Non-normal	Kruskal--Wallis	
Comparison of ranks; PA; C (med: 4) vs M (5); $p < 0.0001$	Non-normal	Dunn's	
Comparison of ranks; PA; C vs H (med: 7); $p < 0.0001$	Non-normal	Dunn's	
Comparison of ranks; PA; H vs M; $p = 0.0908$	Non-normal	Dunn's	
Comparison of ranks; THA; all groups; $p = 0.0028$	Non-normal	Kruskal--Wallis	
Comparison of ranks; THA; C (med: 5) vs M (5); $p > 0.9999$	Non-normal	Dunn's	
Comparison of ranks; THA; C vs H (med: 4); $p = 0.0234$	Non-normal	Dunn's	
Comparison of ranks; THA; H vs M; $p = 0.0071$	Non-normal	Dunn's	
Comparison of ranks; MS 5%; all groups; $p < 0.0001$	Non-normal	Kruskal--Wallis	
Comparison of ranks; MS 5%; C (med: 4) vs M (6); $p < 0.0001$	Non-normal	Dunn's	
Comparison of ranks; MS 5%; C vs H (med: 5); $p = 0.0001$	Non-normal	Dunn's	
Comparison of ranks; MS 5%; H vs M; $p = 0.4328$	Non-normal	Dunn's	
Comparison of ranks; MS 10%; all groups; $p = 0.0731$	Non-normal	Kruskal--Wallis	
Comparison of ranks; MS 10%; C (med: 5) vs M (5); $p > 0.999$	Non-normal	Dunn's	
Comparison of ranks; MS 10%; C vs H (4); $p = 0.1503$	Non-normal	Dunn's	
Comparison of ranks; MS 10%; H vs M; $p = 0.1864$	Non-normal	Dunn's	
Comparison of ranks; IAA 5%; all groups; $p = 0.1561$	Non-normal	Kruskal--Wallis	
Comparison of ranks; IAA 5%; C (med: 7) vs M (7); $p = 0.3965$	Non-normal	Dunn's	
Comparison of ranks; IAA 5%; C vs H (med: 6); $p = 0.1912$	Non-normal	Dunn's	
Comparison of ranks; IAA 5%; H vs M; $p > 0.9999$	Non-normal	Dunn's	
Comparison of ranks; IAA 10%; all groups; $p = 0.3245$	Non-normal	Kruskal--Wallis	

(Continued)

Table 6. Continued

Statistical values	Data structure	Type of test	Power
Comparison of ranks; IAA 10%; C (med: 10) vs M (9); $p = 0.6739$	Non-normal	Dunn's	
Comparison of ranks; IAA 10%; C vs H (med: 9); $p = 0.4643$	Non-normal	Dunn's	
Comparison of ranks; IAA 10%; H vs M; $p > 0.999$	Non-normal	Dunn's	
Comparison of ranks; Hex 5%; all groups; $p < 0.0001$	Non-normal	Kruskal--Wallis	
Comparison of ranks; Hex 5%; C (med: 12) vs M (12); $p > 0.9999$	Non-normal	Dunn's	
Comparison of ranks; Hex 5%; C vs H (med: 12); $p = 0.0012$	Non-normal	Dunn's	
Comparison of ranks; Hex 5%; H vs M; $p < 0.0001$	Non-normal	Dunn's	
Comparison of ranks; Hex 10%; all groups; $p < 0.0001$	Non-normal	Kruskal--Wallis	
Comparison of ranks; Hex 10%; C (med: 9) vs M (12); $p < 0.0001$	Non-normal	Dunn's	
Comparison of ranks; Hex 10%; C vs H (9); $p = 0.7317$	Non-normal	Dunn's	
Comparison of ranks; Hex 10%; H vs M; $p < 0.0001$	Non-normal	Dunn's	
Figure 8			
Habituation; all groups; $p < 0.0001$	Non-normal	Friedman Test	Friedman statistic: 46.52
Habituation; Pre vs 5 min Post; $p < 0.0001$	Non-normal	Tukey's	
Habituation; Pre vs 30 min Post; $p < 0.0001$	Non-normal	Tukey's	
Habituation; 5 vs 30 min Post; $p > 0.9999$	Non-normal	Tukey's	

that prenatal and early postnatal food-based odor exposure increases the number of M/TCs associated with a glomerulus known to be activated by the conditioned odor (Liu et al., 2016). Here, we find that this exposure paradigm also induces widespread changes to odor-evoked MC responses. Such changes may be the result of several mechanisms, including the timing of odor exposure during early development, the use of food-odor association in this paradigm, and/or general sensory enrichment. Further work is necessary to determine the

exact mechanisms resulting in this generalized increase in MC excitability.

Prenatal and early postnatal odorant exposure significantly changes excitatory odor-evoked MC responses in an odor-nonspecific way

The lack of clear odor-specificity in changes following odor conditioning was surprising, given previous work showing specificity in anatomic changes following prenatal and early postnatal odorant exposure (Todrank et al.,

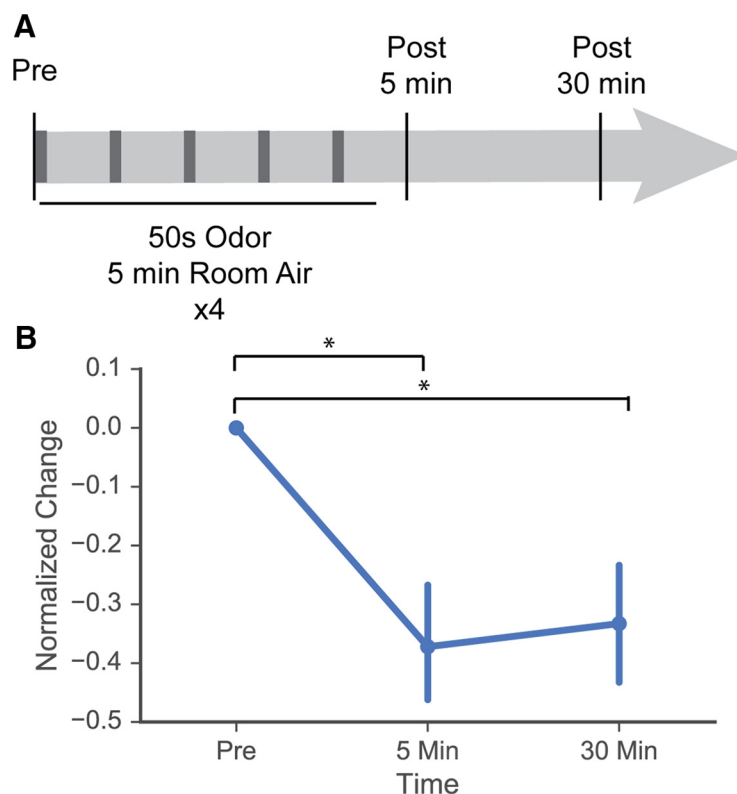


Figure 8. MCs display acute habituation following repeated short odor pulses. **A**, Odor stimulus (hexanal at 1% concentration) was presented for 50 s followed by 5 min of room air, with stimulus repeated four times. MC odor-evoked response was captured before stimulus presentation, 5 min after final 50-s odor stimulus, and 30 min after final 50-s odor stimulus. **B**, Normalized change in odor response between prestimulus and 5-min or 30-min poststimulus. Significant decrease in odor response was observed at both time points poststimulus; * indicates statistically significant comparison.

2011; Liu et al., 2016), previous work showing that an early postnatal conditioning paradigm increases lateral inhibition (Geramita and Urban, 2016), and the observation that early postnatal odor conditioning results in a reduced response to conditioned but not other odors (Wilson et al., 1985). However, 2-DG maps of glomerular activation in rats (Glomerular Activity Response Archive, Michael Leon, gara.bio.uci.edu/index.jsp) show that both MS and hexanal activate a large number of potentially overlapping glomeruli on the dorsal OB surface, so it is possible that the widespread nature of the changes we observe in odor-evoked MC excitatory responses are due to the widespread activation of dorsal glomeruli by these exposure odorants. These changes may not be seen in areas of the OB that lack glomeruli activated by either odor. Although MS and hexanal are quite different perceptually and structurally, the lack of intuitive glomerular organization by odorant in the OB precludes us from knowing whether these two odorants activate very different sets of glomeruli. Further work is necessary to elucidate if these changes in MC response can be generalized to MCs in OB areas known to not be activated by either of these two odorants, or if odorant specific changes in excitability can be achieved with odorants known to have nonoverlapping glomerular activation maps.

Tuning curves of MC response also changed following mint and hexanal odorant exposure. Because there were also significant tuning curve differences between the mint- and hexanal-exposed groups for specific odorants in the stimulus panel, there may be relative changes in response rank specific to the identity of exposed odorant. MS and hexanal have different glomerular activation patterns, thus chronic odorant exposure may change the network of lateral inhibition and change odor-evoked response in overlapping but distinct ways. Use of a larger odor panel including ventral-activating odorants would help elucidate whether these changes in rank and in excitatory responses are connected to the identity of the odorant used for exposure.

In addition, there may be differences in the effect of early odorant exposure on male and female mice. Recent work suggests that OSN signaling differs between female and male mice – specifically, OSNs from female mice respond faster to odor presentation and are more broadly tuned than male OSNs (Kass et al., 2017). However, previous work by Liu et al. (2016) showed that early odorant exposure using the same paradigm as in this study changes the number of M/TCS connected to a single activated glomerulus, but these changes are not significantly different between male and female mice. In our data, we find that the distribution of odor-evoked excitatory MC responses is different between male hexanal-exposed and control mice but not between male mint-exposed and control mice. Contrastingly, there were significant differences in response distribution between female odor-exposed (both hexanal- and mint-exposed) and control mice. However, we only obtained data from one female control and two female odor-exposed animals from each group, insufficient subject number to draw strong conclusions about whether these changes in re-

sponse distribution are due to interanimal variability or sexual dimorphism.

Activation pattern similarity of odorant stimuli

In this study, most cells had observed above-threshold responses to most odorants in the panel (Fig. 5E). This difference from the sparser MC response to odorants observed previously (Kato et al., 2012; Blauvelt et al., 2013; Wachowiak et al., 2013; Roland et al., 2016) could be due to (1) the high sensitivity of GCaMP6s calcium indicator, (2) the choice of odorants within the stimulus panel, (3) the use of higher odorant concentrations in our study, and (4) the use of an anesthetized preparation. In *in vivo* studies of visual cortical neurons, use of GCaMP6s resulted in a fivefold higher rate of detection of responding neurons than GCaMP3, suggesting that the larger proportion of observed odor-evoked responses in our data set could be partially explained by use of a more sensitive calcium indicator (Chen et al., 2013). The choice of odorants used for chronic exposure and in the odor panel was deliberately focused on dorsal OB activating odorants to visualize odor-evoked responses in the cohort of cells imaged, those on the dorsal OB surface. Thus, the composition of the stimulus panel may explain why a large proportion of cells on the dorsal OB surface showed significant odor-evoked responses to many dorsally-activating odorants in the panel.

MC activity differs significantly between the awake and the anesthetized states. Kato et al. (2012), found that the MCs of anesthetized animals are more responsive, each odorant elicits response from a larger number of MCs, and the amplitude of odor-evoked responses is also greater. They also found that experience-dependent habituation of odor-evoked MC response is dependent on odor presentation in the awake state, as brief odor presentation while under anesthesia did not result in MC response habituation. Chaudhury et al. (2010), observed that acute odorant exposure causes habituation of MC response in anesthetized animals, a finding that we replicate in Figure 8. In contrast to Kato et al. (2012), our odorant exposure paradigm consists of food-based odorant exposure throughout gestation and nursing. This paradigm has been shown to induce significant changes in both glomerulus volume and the number of M/TCS connected to single glomeruli (Todrank et al., 2011; Liu et al., 2016). Todrank et al. (2011), additionally observed that odorant exposure limited to either the gestation period or the postnatal nursing period induced an increase in glomerular volume. In this study, we do not address whether the experience-dependent changes in MC response can be observed following odorant exposure limited to a specific developmental period or whether these changes can be observed in the awake animal. However, given the significant anatomic changes in the OB observed after prenatal and early postnatal odorant exposure, we predict that we would also observe an increase in excitatory odor-evoked MC response in awake mice and that this change remains robust following exposure limited to either gestation or nursing.

Prenatal and postnatal timing of odorant exposure

The timing of our odorant exposure paradigm may be one explanation for why we find an increase rather than a decrease in excitability following exposure. Here, we use a conditioning paradigm of constant odor exposure during both gestation and the postnatal period. [Todrank et al. \(2011\)](#) show that exposure during either gestation or early nursing is sufficient to significantly increase the size of activated glomeruli, while [Liu et al. \(2016\)](#) demonstrate that this paradigm also increases the number of M/TCs connected to a single activated glomerulus. Prenatal food-based odor exposure can produce large anatomic changes in the OB circuitry. We show that this paradigm also promotes nonspecific changes to excitatory MC odor-evoked responses; these findings contrast with previous studies of odor-evoked MC responses following both acute and chronic odor exposure. However, the majority of studies analyzing experience-dependent changes to the structure and function of the OB rely on postnatal manipulations ([Benson et al., 1984](#); [Laing, 1985](#); [Panhuber and Laing, 1987](#); [Saghatelian et al., 2005](#); [Kerr and Belluscio, 2006](#); [Woo et al., 2006](#); [Cavallin et al., 2010](#); [Johnson et al., 2013](#); [Morrison et al., 2015](#); [Geramita and Urban, 2016](#)). Starting exposure during gestation may trigger developmental changes distinct from those observed with postnatal odor experience.

Sensory enrichment

Generalized early postnatal sensory enrichment may be another explanation for the observed nonspecific enhanced excitatory responses. Odor deprivation during development causes significant changes to OB structure and activity. OB size, OSN activity, MC connectivity, and granule cell integration are all impacted by early nares occlusion ([Benson et al., 1984](#); [Saghatelian et al., 2005](#); [Cavallin et al., 2010](#)). With regards to the anatomic effects of early postnatal chronic odor enrichment or stimulation, the consensus is less clear. A number of studies report a significant decrease in the size and density of MCs following chronic passive odor exposure ([Laing, 1985](#); [Panhuber and Laing, 1987](#); [Woo et al., 2006](#); [Johnson et al., 2013](#)). However, [Rosselli-Austin and Williams \(1990\)](#), used scented objects in a normal cage setting to deliver chronic odor stimulation and found that mitral and granule cell numbers actually increased following neonatal odorant exposure. In addition, numbers of adult born granule cells ([Rochefort et al., 2002](#)) and dopaminergic cells ([Bonzano et al., 2014](#)) also increase following chronic postnatal odorant exposure. These results suggest that richness of the sensory environment during the neonatal period can significantly modify OB structure. Our work points to a general increase in MC excitability, as measured by the number and amplitude of excitatory odor-evoked responses. This generalized change could be due to sensory enrichment through odorant exposure during a critical period of OB development.

Enrichment can also modify the excitability of other sensory systems. Studies in cat visual cortex showed that rearing animals in an enriched environment increased the number of orientation selective cells, sharpened orienta-

tion tuning of cells, and increased responsivity of cells to light stimuli ([Beaulieu and Cynader, 1990a,b](#)). Such changes are observed during adulthood as well, following visual enrichment, adult rats with amblyopia demonstrated improved visual acuity and reduced inhibition/excitation balance in V1 ([Baroncelli et al., 2012](#)). Other sensory systems also demonstrate this feature, with sensory enrichment increasing excitatory responses and refinement of stimulus selectivity within the auditory and somatosensory systems ([Coq and Xerri, 1998](#); [Bourgeon et al., 2004](#); [Engineer et al., 2004](#); [Alwis and Rajan, 2013](#)). This phenomenon also may be taking place in the OB, as we observe a generalized, nonstimulus specific increase in MC excitatory response amplitude and number following odorant exposure.

Food-associated exposure paradigm

Lastly, food-association may contribute to the generalized increase in MC excitation. Here, we pair food with odorant. For the moth *Manduca sexta*, pairing repeated odor exposure with food resulted in an increase in responsive neurons while repeated odor exposure without food caused a decrease in responsive neurons ([Daly et al., 2004](#)). While these experiments were conducted in a different organism and used acute trials of odor pairing rather than the chronic odor exposure that we use, the *Manduca* study demonstrates that odorant context plays a role in experience-dependent modifications in response. When exposed to odorized food, mice demonstrate a distinct preference for food scented with the familiar odorant ([Todrank et al., 2011](#); [Liu et al., 2016](#)), indicating a positive odorant association following food-based exposure. However, [Wilson et al. \(1985\)](#), found that pairing odor puffs with brushing, an association with a positive or attractive context, results in a decrease of excitatory responses to presentations of the exposure odorant alone. The contrasting results from studies that use different odor exposure paradigms, reward-paired or passive, suggest that context of odor presentation could play an important role in determining how the circuit remodels anatomically and functionally following repeated or chronic odor exposure.

Conclusion

Here, we use a food-based paradigm to investigate the effects of prenatal and early postnatal odor exposure on odor-evoked responses of MCs. We find that odorant exposure heightened dorsal OB MC activity, increasing the amplitude, reliability, and the proportion of excitatory odor-evoked MC responses. We also find that odorant exposure changed the tuning curves of MCs in exposed animals. These effects were not specific to odor-evoked responses to either MS or hexanal, the odorants used for exposure. Rather, prenatal and early postnatal odorant exposure using either odorant resulted in generalized changes to MC responses across the dorsal OB and in response to all odorants in the stimulus panel. Control, hexanal-, and MS-exposed animals all demonstrated a similar decrease in odor-evoked MC responses following acute odorant habituation with hexanal, indicating that

our paradigm of constant early odorant exposure does not fundamentally change mechanisms of habituation.

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