Chemical Senses, 2021, Vol 46, 1–15
doi:10.1093/chemse/bjaa071
Original Article
Advance Access publication 26 October 2020

Original Article

Olfactory Deprivation and Enrichment: An Identity of Opposites?

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Editorial Decision 20 October 2020.

Abstract

The effects of deprivation and enrichment on the electroolfactogram of mice were studied through the paradigms of unilateral naris occlusion and odor induction, respectively. Deprivation was shown to cause an increase in electroolfactogram amplitudes after 7 days. We also show that unilateral naris occlusion is not detrimental to the gross anatomical appearance or electroolfactogram of either the ipsilateral or contralateral olfactory epithelium even after year-long survival periods, consistent with our previous assumptions. Turning to induction, the increase in olfactory responses after a period of odor enrichment, could not be shown in CD-1 outbred mice for any odorant tried. However, consistent with classical studies, it was evident in C57BL/6J inbred mice, which are initially insensitive to isovaleric acid. As is the case for deprivation, enriching C57BL/6J mice with isovaleric acid causes an increase in their electroolfactogram response to this odorant over time. In several experiments on C57BL/6J mice, the odorant specificity, onset timing, recovery timing, and magnitude of the induction effect were studied. Considered together, the current findings and previous work from the laboratory support the counterintuitive conclusion that both compensatory plasticity in response to deprivation and induction in response to odor enrichment are caused by the same underlying homeostatic mechanism, the purpose of which is to preserve sensory information flow no matter the odorant milieu. This hypothesis, the detailed evidence supporting it, and speculations concerning human odor induction are discussed.

Key words: anosmia, EOG, induction, naris occlusion, plasticity

Introduction

For studies of neural plasticity, no experimental manipulations have been more informative or frequently employed than sensory deprivation and enrichment. Their use has illuminated the role of activity-dependent processes—both permissive and instructive—in maturing and adult nervous systems through research spanning several generations. From the classical studies of Hubel and Wiesel (1998, 2005) who used monocular deprivation in cats to demonstrate a critical period for ocular dominance plasticity to the revisionist studies of Merzenick et al. (Zhang et al. 2002; Zhou et al. 2011) and Gilbert et al. (2001) who used (among other methods) sensory enrichment to demonstrate adult cortical plasticity, these extreme opposite manipulations have been indispensable.

Also for the olfactory system, deprivation and enrichment have been mainstays to those interested in experience dependence. If anything, the potential of this sensory channel to remain malleable throughout life has drawn enhanced research interest owing to ongoing neurogenesis in primary and secondary neuron populations (Graziadei and Monti-Graziadei 1978; Brann and Firestein 2014).

Although enrichment methods have been varied, deprivation in olfactory studies has predominantly relied on unilateral naris occlusion (UNO). In this technique, which has been used in experimental studies of the olfactory system for over a century, one nostril is surgically or mechanically obstructed to impede the flow of odorant-carrying air into the ipsilateral nasal cavity (Gudden 1870). Since there is little exchange of air or crossing of olfactory information

between the 2 sides of the nasal cavity, this manipulation provides, in theory, a convenient comparison between a stimulated and unstimulated (or at least less stimulated) olfactory circuit.

In the developing rodent, a panoply of UNO effects—at the molecular, biochemical, circuit, and behavioral levels—have come to light in the last several decades, too numerous and far-flung to review here. In brief compass, earlier studies tended to emphasize the deleterious effects ("use it or lose it") of UNO on the ipsilateral olfactory circuit (Brunjes 1994), whereas more recent studies have tended to emphasize compensatory responses to this form of deprivation (Coppola 2012).

Adults have been less-frequent targets of olfactory deprivation studies, but effects on the ipsilateral olfactory pathway following early postnatal UNO have been shown to accrue in adults as well including reductions in bulb size (Henegar and Maruniak 1991); neurogenesis (Mandairon et al. 2006); and granule cell branching (Dahlen et al. 2011). In a previous study from our laboratory, removable nasal plugs were used to demonstrate that seemingly identical compensatory responses to deprivation described for developing mice also occur in adults (Barber and Coppola 2015). Taken together, the studies of the effects of UNO on the adult olfactory system can also be divided into those documenting losses of function and those documenting gains (Brunjes 1994; Coppola 2012).

Concerning enrichment, single odorant exposure during the perinatal period has been linked to changes in abundance of the corresponding olfactory transcripts (Cadiou et al. 2014; Dewaelea et al. 2018), increases in number and size of the corresponding glomeruli (Todrank et al. 2011; Valle-Leija et al. 2012; Dewaelea et al. 2018), changes in olfactory bulb cellular abundance and electrophysiological responses (Wilson et al. 1985; Woo and Leon 1987; Rosselli-Austin and Williams 1990; Liu et al. 2016), and postnatal behavior preferences for the exposure odorant (Smotherman 1982; Nolte and Mason 1995; Hudson and Distel 1998; Todrank et al. 2011, Dewaelea et al. 2018) to list but a few findings. However, inconsistencies in this literature abound with many of the reported effects of enrichment contradicted by other studies (Kerr and Belluscio 2006; Cavallin et al. 2010; Cadiou et al. 2014; Dewaelea et al. 2018).

As was the case for deprivation, adults have been less-frequent targets of odorant enrichment studies than developing animals. One of the main areas of interest in adults has been the effect of odorant enrichment on the birth, survival, and functional integration of adult-born neurons, which repopulate the olfactory bulbs throughout life (Rochefort et al. 2002; Rey et al. 2012; Moreno et al. 2014). It is in the adults that investigators have repeatedly observed one of the most striking, intriguing, and still mysterious forms of olfactory plasticity referred to here as "induction" after Wysocki et al. (1989) who first substantiated it for the steroid odorant androstenone. In its classic form, induction involves anosmics: people (or animals) who are unable to smell a particular odorant but who otherwise have a normal sense of smell (Amoore 1967). In a portion of these anosmic subjects, the ability to smell androstenone is spontaneously induced by repeated exposure. In practice, this process is documented by changes in threshold, which can be quite dramatic and take place after a surprisingly small amount of exposure (Wysocki et al. 1989). Relevant to the work to be described here, subsequent research discovered inbred mouse models of anosmia, which display induction-like responses to strain-specific odorants (Wang et al. 1993).

Despite the more than 3 decades that have elapsed since the first detailed description of induction, its fundamental nature remains an enigma. Is it a form of perceptual learning instantiated in central circuits or might it be a novel form of peripheral sensitization? Does it only occur for a few odorants or is it general? How long does induction persist? Is the phenomenon that exists in humans the same as that described in rodents? Does induction offer any advantage or is it, perhaps, a laboratory artifact? And finally, what is induction's relationship with deprivation, if any. The following studies in different mouse strains that compare the electrophysiological responses of the olfactory epithelium (OE) to odorants after deprivation and enrichment shed light on some of these questions and support the conclusion that deprivation and enrichment may be more related than previously appreciated.

Materials and methods

Animals

Animal procedures, including husbandry and experimental manipulations, were approved and supervised by the Randolph-Macon College Institutional Animal Care and Use Committee and complied with the *Guide for the Care and Use of Laboratory Animals* (8th Edition, National Academies Press). Mice were kept in an approved animal room maintained on a 12 h/12 h light cycle with standard mouse chow available ad lib. Subjects were either females of the CD-1 outbred strain obtained from Charles River Laboratories at 56–60 days of age and used in the study up to 1 year of age or females of the C57BL/6J (C57) inbred strain obtained from Jackson Laboratories at 56–60 days of age and used up to 7 months of age.

Naris plugs

Deprivations was achieved using unilaterally inserted naris plugs. Removable naris plugs were constructed according to the procedures of Cummings et al. (1997). Briefly, plugs consisted of 4-mm sections of 0.96-mm-OD polyethylene tubing occluded with a knotted piece of 3.0 silk. A single strand of human hair was tied into the suture knot and trimmed to protrude 1 mm from one end of the tubing. The hair facilitated plug positioning and removal. Plugs were lubricated with ophthalmic ointment and inserted in either the right or left naris, while the animal was under brief isoflurane anesthesia. Animals were removed from the study if their plugs became dislodged or if the application of soap bubbles at the time of animal use revealed any airflow through the plugged naris.

Surgical naris occlusion

Permanent naris occlusion followed the procedures of Coppola et al. (2006) and Barber and Coppola (2015). Briefly, on the day after birth pups were anesthetized with light isoflurane inhalation followed by hypothermia. One of the 2 nares was occluded by using a surgical cautery to cut a slot across the naris that was then filled with cyanoacrylate glue. Mice were excluded from the study if their occluded naris was patent at the time of use in the study.

Odorant enrichment

Different methods of odorant enrichment were used depending on the experiment. For the first experiments with R-carvone (CAR), acetophenone (ACP), and isoamyl acetate (IAA) on CD-1 strain mice, enrichment was effected by mixing odorants in the drinking water at a 1 mM concentration and placing a standard "tea ball" (approximately 3 cm diameter perforated stainless steel ball) in the home cage floor. These methods were chosen because they have been shown to be effective in previous enrichment studies (Todrank et al. 2011; Cadiou et al. 2014; Ibarra-Soria et al. 2017). The tea ball contained 100 µl of pure odorant adsorbed onto a cotton ball and was in the cage twice

per day for 1 h each, between the hours of 8:00 AM and 7:00 PM with 5 h separating the exposures. In an experiment termed "late onset" enrichment, mice were exposed to test odorants as adults (>55 days) for 20 days. In a second experiment termed "early onset," only CAR and ACP were used. Exposure, in drinking water and tea ball, started on the day of birth and continued for 56–58 days. In this latter case, we cannot be certain that newborn mice were exposed to the odorant in the dam's drinking water as it may have been metabolized before reaching the pups through her milk.

For most of the isovaleric acid (IVA) induction experiments, that involved both C57 and CD-1 mice, the methods of Wang et al. (1993) were carefully replicated according to their published description. Consequently, odorant enrichment was accomplished by placing a cotton gauze soaked with 10^{-2} M aqueous solution of IVA (>99% purity, Sigma–Aldrich) on the wire top of a standard mouse cage. The cage was then "loosely covered" with a polyethylene sheet to "concentrate" odorant (Wang et al. 1993). The gauze stayed in place for 16 h/day starting a 4:00 PM and ending at 8:00 AM. Control mice were kept in a separate room from the experimental group but otherwise were treated identically except that no odorant was placed on the gauze. The enrichment period lasted between 14 and 40 days. Cotton gauzes were removed from the subjects' cages 24 h before electroolfactograms (EOGs) were performed.

The enrichment methods for the final IVA exposure duration experiment (3 days vs. 7 days) varied from those of Wang et al. (1993). In this experiment, an outlet timer was used to control a standard aquarium pump that passed room air at 1.28 l/min through a 1-L flask that contained 700 mL of 10^{-2} M aqueous solution of IVA. The output tube of the flask was affixed to the top of the mouse cage so that odorized air suffused its interior. Plastic sheeting was used, as above, to concentrate odorant. The control group for this experiment was treated identically except that the "odorant" flask contained only distilled water. The timers were set to turn the pumps on between 4:00 PM and 8:00 AM daily for either 3 or 7 days. Odorant and control air delivery ceased 24 h before EOGs were recorded.

Stimulus set

Odorants were commercially available chemicals generally at the highest purity available (Sigma–Aldrich). IAA was used in the nose plug experiment because it had been proven effective in a previous study (Barber and Coppola 2015). ACP and CAR are widely used in olfactory studies and had been used in our previous EOG and behavioral studies (Barber and Coppola 2015; Coppola et al. 2017, 2019; Blount and Coppola 2020). IVA and IAA were used in the induction experiments because their detection had previously been shown to be inducible in mice (Wang et al. 1993; Voznessenskaya et al. 1995). Octanoic acid (OA) was used in the induction study because of its chemical similarity to IVA and because we had experience using this chemical in a previous EOG study (Coppola et al. 2019).

EOG recordings

Our EOG methods have been thoroughly and repeatedly described previously (Waggener and Coppola 2007; Coppola et al. 2013, 2017; Barber and Coppola 2015) and will only be briefly described here. Prior to electrophysiological recording, mice were administered a lethal dose of Euthasol (70 mg/kg i.p.), which has been shown not to alter EOG responses (Scott and Scott-Johnson 2002). Immediately after death, subjects were decapitated and their skulls were bisected along the midsagittal plane. Both the right and left nasal cavities were used for recording responses to odorants with ordering of side counterbalanced. Final preparations entailed resection of the nasal septum and overlying mucosa to expose the medial aspect of the endoturbinates. Two locations on the dorsal branch of endoturbinate 2 (referred to herein as simply II) and one location each on endoturbinate III and IV were targeted for EOG recording (Figure 1). These location were chosen either because of our experience with their response profile from our previous studies for which we established a standard grid of mucosal recordings sites (Coppola et al. 2017) or because the location was used in a previous study by Wang et al. (1993).

Recordings took place within a Faraday cage covered with plastic sheeting and suffused with the output of 2 commercial humidifiers to maintain the preparation >98% humidity. The humidifiers also created positive pressure that exhausted spent odorants from the chamber.

Recording electrodes consisted of Ag/AgCl wires inside glass capillaries pulled to approximately 50-µm tip diameter and filled with 0.05% agar in 0.1 M phosphate-buffered saline. To record the EOG, an electrode was positioned at a desired location using a 3-axis manipulator. An indifferent electrode was placed on the frontal bone at its intersection with the cribriform plate and immobilized with a magnetic clamp. The indifferent electrodes consisted of Ag/AgCl wire inside a 500-µl pipette tip filled as above. Electrodes were connected to the inputs of an Iso-DAM8A DC Amplifier (World Precision Instruments). The output of the amplifier was sampled at 20 kHz by a PowerLab/8SP physiograph (AD Instruments), which provided A/D conversion, display, and recording. The absolute value of the EOG maximum amplitude was the dependent variable for all experiments and was measured by manual cursor placement at the nadir of the EOG trace using LabChart software (v7.2.5).

Once the electrode was positioned at a desired location testing proceeded from lowest to highest stimulus concentration however

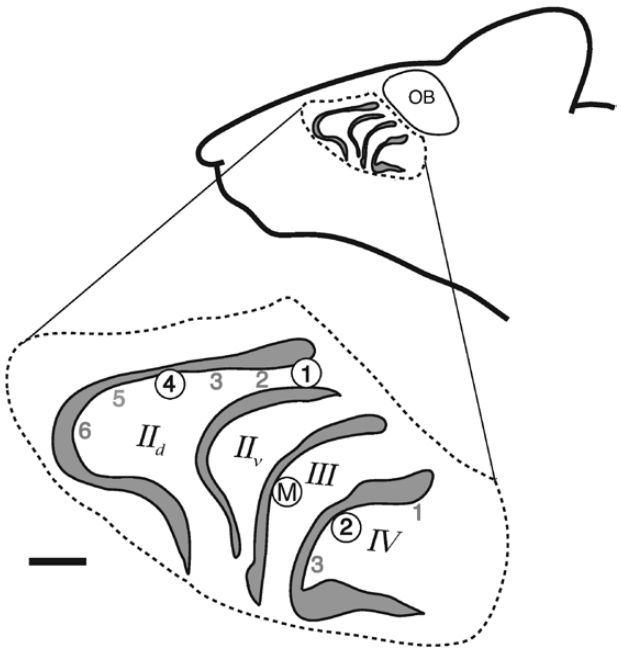

Figure 1. Midsagittal drawing of endoturbinates showing EOG recording sites used in this study (circles) that are mapped onto our standard recording locations (Coppola et al. 2017). Roman numerals designate different endoturbinates. Note there are sites on the dorsal branch of endoturbinate II and on III, and IV. The site designated with an M is the middle position along the curvature of turbinate III matching a recording site of Wang et al. (1993). Scale bar = 1 mm.

the ordering of odorant type and turbinate location was counterbalanced across animals. Each combination of stimulus odorant and concentration was tested once at each recording site.

The odorant delivery port consisted of a 3-cm-long, 3.5-mm-diameter glass tube connected by a 3-cm-long Teflon tube to the odorant reservoir vial. A 3-axis micromanipulator was used to position the odorant port. A rigid guide-hair affixed to the end of the odorant port allowed us to set a consistent standoff distance of 10 mm and an angle of 45 degrees normal to the surface of the OE.

Odorants were delivered to the mucosal surface via a 0.5-s pulse of air (700 mL/min) from the headspace of a 25-mL vial containing 10-mL mixture of odorant dissolved in mineral oil. Charcoal-filtered and humidified room air served as the carrier gas. A custom unit (Knosys Inc.) consisting of computer, software, interface, and olfactometer-controlled stimulus duration and timing. Stimulus odorant and concentration were selected by manually switching the reservoir vial that was in line with the odorant port. Interstimulus interval was held to a minimum of 50 s, and all recordings were completed within 45 min of death.

All recordings were performed by an investigator who was blind to the treatment conditions.

Statistical analyses

For all experiments the α level was set at $P < 0.05$, and only a prior hypotheses were tested. Sample sizes were chosen based on our previous EOG studies (Waggener and Coppola 2007; Barber and Coppola 2015. Analyses were performed in Prism Ver. 8.4.3 (GraphPad). To accommodate the combination of repeated measures (odorant or concentration) and independent samples (deprivation or enrichment status), mixed-model 2-way ANOVAs were computed. When necessary, these calculations were followed by an appropriate multiple comparison test. The Geisser–Greenhouse correction was used leading to nonwhole number degrees of freedom. Spearman's correlations were calculated in the experiment that focused on the relationship between enrichment duration and induction. Because different areas of the OE can have variable responses to the same stimulus, recording sites were analyzed independently. However, recording location ended up having little bearing on study conclusions.

Results

Onset timing of compensatory response

The onset timing of olfactory compensatory plasticity was studied by placing nose plugs in groups of adult CD-1 mice for 24 h, 3 days, 7 days, 10 days, or 13–16 days. Following these survival periods, EOG responses to a 0.1% v/v concentration of IAA were measured from the open and occluded sides of the nasal cavity targeting turbinate II, location (Loc) 1 and 4 (Figure 1). To test onset dynamics statistically, we performed a mixed-model, 2-way ANOVA followed by Sidak's multiple comparison test (Figure 2; Prism, GraphPad). For recording Loc 1, there was not a statistical difference between the survival periods overall ($F_{4,39} = 2.27$; $P > 0.08$) even though the mean EOGs from the longest survival period appear to be smaller and less variable than the other survival times. However, there was a highly significant effect of nasal plugs ($F_{1,39} = 30.5$; $P < 0.0001$) and a plug-condition versus survival-period interaction ($F_{4,39} = 3.1$; $P < 0.03$). Sidlak's multiple comparison test probabilities show that by the 7-day survival point and beyond the plugged-side displayed larger magnitude EOGs (Figure 2A). The results were similar for recording Loc 4. EOG responses after different survival periods were not statistically different pooled across plug conditions ($F_{4,39} = 2.4$; $P > 0.06$) though for unknown reasons the mean EOGs for the longest survival period appear to be smaller and less variable than the other survival time. Again, there was a highly significant effect of nasal plugs ($F_{1,39} = 15.5$; $P < 0.0003$) and a significant plug-condition versus survival-period interaction ($F_{4,39} = 3.1$; $P < 0.03$). Sidlak's test resulted in significant probabilities for the effect of naris plugging beginning at the 7-day survival period and beyond (Figure 2B). That these effects were due to an increase in the occluded-side responses and not a decrease in open-side responses is borne out by the relative uniformity of the open side responses at all the survival periods.

Long-term naris occlusion

The following results are from an experiment born of opportunity. Two female and one male CD-1 mice were naris occluded on the first day after birth and held in our animal facility for over a year (379 or 380 days). They were originally part of another study but were never needed. However, their long survival—unexplored in our previous studies of naris occlusion—allowed us to determine if UNO has a deleterious effect after extended periods on either the open side or occluded side of the nasal cavity.

At the level of gross anatomy, the open- and the occluded-side endoturbinates and mucosal covering were surprisingly similar (Figure 3). However, for each animal, the open-side mucosa appeared more heavily pigmented and the endoturbinates appeared more robust, consistent with previous observations of the effects of shorter-term occlusion (Coppola et al. 2014). EOGs were recorded from endoturbinate II, Loc 4 (Figure 1) in response to 3 concentrations of isoamyl acetate: 0.004%, 0.02%, and 0.1% (Figure 3, traces). Perhaps surprisingly given the long survival period (380 days), EOG responses from both the open and occluded side of the nasal cavity appeared normal in onset and recovery dynamics if slightly lower in magnitude compared to mice in our typical age range (2–6 months). For two of the mice (females F18B and F18C), EOG magnitudes were consistently larger on the occluded side compared with the open side; the reverse was true for the third mouse (male M18A). These observations, though limited by lack of replication, suggest that even very long-term UNO is not overtly deleterious to the either the ipsilateral or contralateral olfactory mucosa. Furthermore, these data do not rule out the possibility that the enhancement of EOG responses on the occluded side observed after shorter survival periods may be quite long-lived.

Adult and early-onset enrichment

Adult CD-1 mice received ACP, CAR, or IAA odorant enrichment for 20 days as described in Materials and methods. EOGs were then recorded in response to 0.1% v/v and 0.02% v/v concentrations of these odorants at locations: turbinate II, Loc 4 and turbinate IV, Loc 2 (Figure 1). For the 0.1% stimulus concentration at recording site turbinate II, Loc 4 (Figure 4A), odorant type had a significant effect on the mean EOG ($F_{1.8, 38.6} = 62.4$, $P < 0.0001$), but enrichment did not ($F_{2, 21} = 0.84$, $P > 0.4$) and the interaction term was not significant ($F_{4, 42} = 1.1$, $P > 0.3$). Similar results were found for the recording site turbinate IV, Loc 2 (Figure 4B): Odorant had a significant effect on the mean EOG ($F_{1.7, 36.4} = 36.2$, $P < 0.0001$) but enrichment did not ($F_{2, 25} = 0.16$, $P > 0.8$). However, in this case, the odorant and enrichment interaction term was significant ($F_{4, 42} = 6.0$, $P < 0.001$).

For the 0.02% stimulus concentration at recording site turbinate II, Loc 4 (Figure 4C), odorant type did not have a significant effect

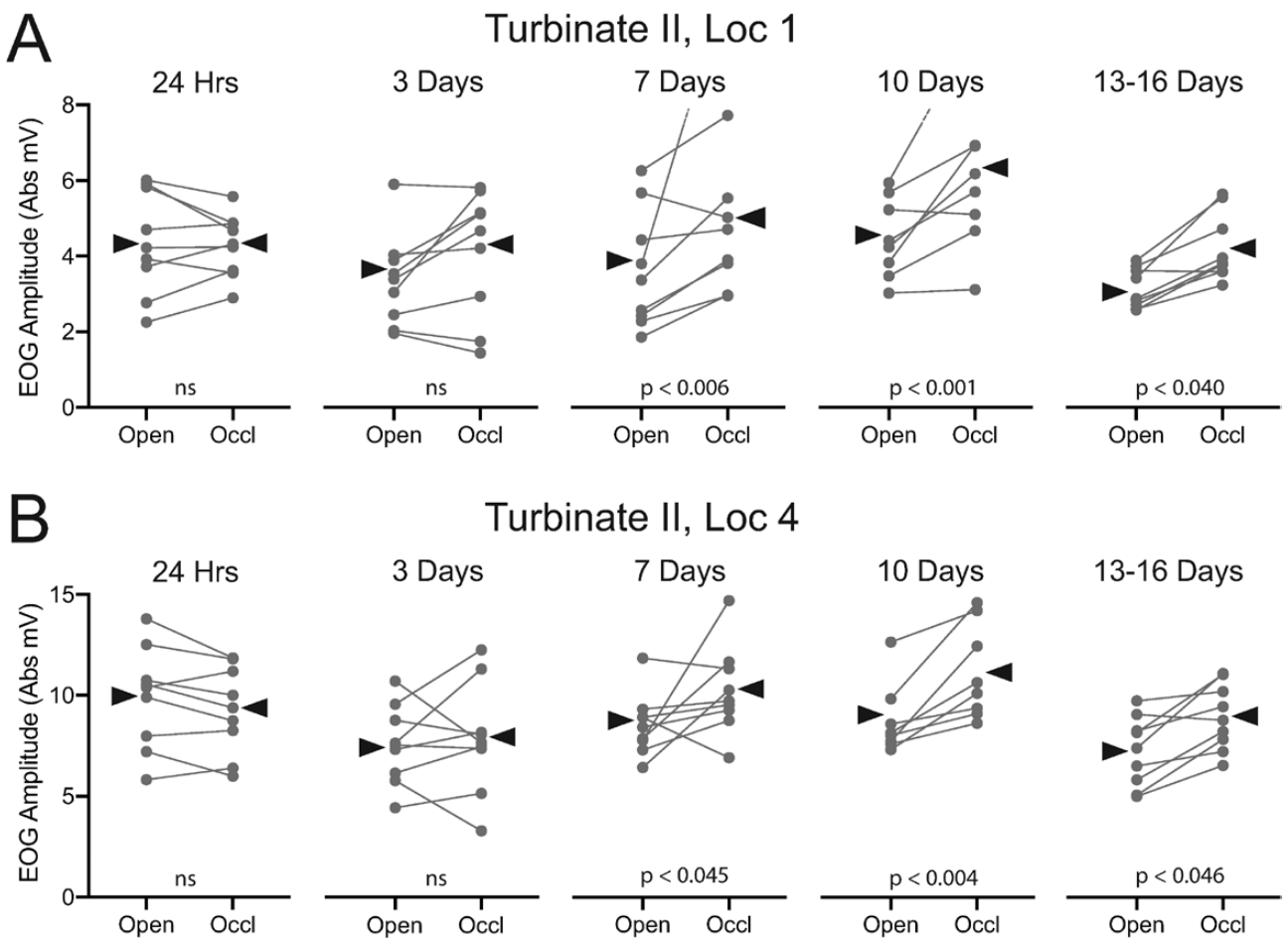

Figure 2. Absolute values of the EOG amplitudes in response to stimulation by a 0.1% v/v concentration of IAA from mice with unilateral nasal plug. Filled circles show responses by OE of individual mice that wore plug for different periods of time. Lines connect responses from the open and occluded (Occl) sides from the same mouse (arrow heads show means; $n = 8$ or 9 for each time period). (**A**) Data from turbinate II, Loc 1 (see Figure 1). (**B**) Data from turbinate II, Loc 4 (see Figure 1). Both (A) and (B) show Sidlak's multiple comparison test probabilities for differences between open and occluded side data (see text for statistical details).

on the mean EOG ($F_{1.6, 33.6} = 1.95$, $P > 0.16$) nor did enrichment ($F_{2, 21} = 1.34$, $P > 0.28$), and the interaction term was not significant ($F_{4, 42} = 0.47$, $P > 0.76$). For the 0.02% stimulus concentration at the turbinate IV, Loc 2 recording site (Figure 4D) odorant emerged, again, as a significant factor in the ANOVA of EOG magnitude ($F_{1.7, 36.8} = 15.9$, $P > 0.0001$) but enrichment did not ($F_{2, 21} = 0.1$, $P > 0.93$) nor was there a significant interaction between odorant and enrichment ($F_{4, 42} = 0.80$, $P > 0.5$).

Considering the adult onset enrichment data together, mean EOG responses varied across odorants and locations consistent with our previous mapping studies (Coppola et al. 2013, 2017). At least for the 0.1% odorant concentration, ACP tended to evoke the largest response at both recording locations while CAR evoked the lowest response (Figure 4). The diminution of this relationship at the 0.02% stimulus concentration suggests that we were approaching the lower limit of the EOG response range which never drops to zero owing to the mechanical response caused by puffing air on the mucosa (Grosmaitre et al. 2007).

Despite these clear and repeatable differential responses, the data do not provide even a suggestion of an effect of enrichment for any odorant-location-concentration combination. We reasoned that this may be due to the adult onset of the enrichment or perhaps that the enrichment lasted for an insufficient time. To investigate these factors a smaller group of mice were enriched with either ACP or CAR from the day after birth until they were 56–58 days of age (see Materials and methods). The stimulus set was expanded to include 0.008%, 0.004%, 0.002%, and 0.1% v/v concentrations of odorant and EOGs were measured as previously.

For the turbinate II, Loc 4 recording site and ACP stimulus (Figure 5A), concentration, as expected, had a significant effect on mean EOG magnitude ($F_{3, 21} = 102.6$, $P < 0.0001$) but enrichment odorant was not significant ($F_{1, 7} = 0.27$, $P > 0.62$), nor was the interaction term ($F_{3, 21} = 0.34$, $P > 0.79$). For the turbinate IV, Loc 2 recording site and ACP stimulus (Figure 5B), concentration was a significant factor ($F_{3, 21} = 83.0$, $P < 0.0001$), but neither enrichment odorant ($F_{1, 7} = 1.04$, $P > 0.34$) nor the interaction term ($F_{3, 21} = 1.01$, $P > 0.40$) were significant.

Similar results were obtained when CAR was the stimulus used. For the turbinate II, Loc 4 recording site and CAR stimulus (Figure 5C), concentration, again, had a significant effect on mean EOG magnitude ($F_{3, 21} = 24.1$, $P < 0.0001$) but enrichment odorant was not a significant factor ($F_{1, 7} = 2.05$, $P > 0.19$), nor was the interaction term ($F_{3, 21} = 0.19$, $P > 0.90$). For the turbinate IV, Loc 2 recording site and CAR stimulus (Figure 5D), concentration was a significant factor ($F_{3, 21} = 20.2$, $P < 0.0001$) but neither enrichment odorant ($F_{1, 7} = 0.96$, $P > 0.35$) nor the interaction term ($F_{3, 21} = 0.51$, $P > 0.67$) were significant.

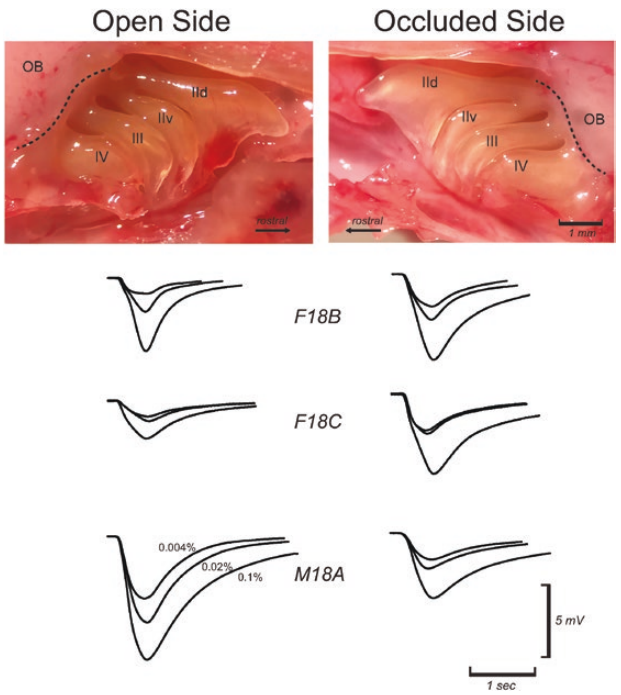

Figure 3. Top: Micrographs of midsagittal views of endoturbinates from the open side (left) and occluded side (right) of a mouse that had been unilaterally occluded for more than 1 year. Roman numerals as in Figure 1; OB, olfactory bulb. Note more robust turbinates on open side. **Bottom**: EOG traces in response to different concentration of IAA (0.004%, 0.02, 0.1% v/v) from open and occluded sides of 3 different mice that had been unilaterally occluded for more than 1 year. Sensitivity, onset and offset kinetics appear normal on open and occluded sides of the nasal cavity.

Taken together, neither earlier-onset nor longer-duration caused ACP and CAR odorant enrichment to have a significant effect on the amplitude of EOG responses to ACP and CAR over most of the OE response range.

IVA induction

The contrast of the clear and repeatable effects of olfactory deprivation by naris occlusion with our inability to demonstrate any effect of enrichment prompted us to attempt to replicate a classic study of induction through odorant enrichment by Wang et al. (1993). In this study, EOG response amplitudes were larger compared to controls in the C57 inbred mouse strain after 2 weeks of enrichment with IVA, an odorant to which this strain is relatively insensitive (Wysocki et al. 1977; Wang et al. 1993).

Examples of EOG recordings from a control mouse and an IVA enriched mouse from our partial replication of Wang et al. (1993) illustrate what was a general result (Figure 6). At all concentrations, mice enriched with IVA for at least 2 weeks tended to display larger magnitude EOGs in response to IVA but not IAA demonstrating an odorant-specific induction effect. Summary data from 2 recording locations validate these visual impression (Figure 7). For the turbinate II, Loc 1 recording site and IVA stimulus (Figure 7A) odorant concentration was a significant factor ($F_{2.4, 43.3} = 90.1$, $P < 0.0001$) as expected. More importantly, enrichment was also a significant factor ($F_{1, 18} = 52.2$, $P < 0.0001$) as was the interaction of concentration and enrichment condition ($F_{3, 54} = 20.6$, $P < 0.0001$). However, for the same recording site when IAA was the stimulus (Figure 7B), though concentration was a significant factor ($F_{1.89, 34.1} = 64.2$, $P < 0.0001$) enrichment was not ($F_{1, 18} = 0.04$, $P > 0.84$) and the interaction between concentration and enrichment was also not significant ($F_{3, 54} = 0.54$, $P > 0.65$).

At turbinate III, Loc M these results were largely replicated. When IVA was the stimulus (Figure 7C) concentration was a significant factor ($F_{2.35, 42.3} = 42.27$, $P < 0.0001$) as was enrichment ($F_{1, 18} = 21.3$, $P < 0.0002$), but the interaction between concentration and enrichment was not significant ($F_{3, 54} = 2.03$, $P > 0.12$). When IAA was the stimulus (Figure 7D), concentration was a significant factor ($F_{1.49, 26.9} = 66.8$, $P < 0.0001$), but enrichment was not ($F_{1, 18} = 0.04$, $P > 0.85$) nor was the interaction between concentration and enrichment ($F_{3, 54} = 0.27$, $P > 0.84$).

In the analyses above, results from all the exposure durations between 14 and 40 days were pooled into a single group. To examine a possible relationship between duration of enrichment and magnitude of induction, EOGs from the enrichment group were expressed as a percent of control responses (averaged within concentration and recording location) and plotted against exposure duration (data not shown). For recording site turbinate II, Loc 1 the mean magnitude of the induction summed over all concentrations was 159.7% of the control mean. However, exposure duration was not correlated with the induction magnitude for any concentration (Spearman's *r*: $P > 0.7$, 0.5, 0.5, and 0.13 lowest to highest concentration). For the recording site turbinate III, Loc M, the mean magnitude of the induction summed over all concentrations was 167.6% of the control mean. Again, exposure duration was not correlated with the induction magnitude for any concentration (Spearman's *r*: $P > 0.7$, 0.7, 0.3, and 0.8 lowest to highest concentration). Although the sample size was modest for this analysis, $n = 10$ enriched mice, the fact that 7 of the 8 correlations (2 recording locations and 4 stimulus concentrations) were negative, suggest that if there is any relationship between exposure duration and induction magnitude it may be negative beyond 2 weeks of exposure.

Induction specificity

Having demonstrated induction with an inbred mouse strain using the protocol by Wang et al. (1993), we sought to extend these finding. Following the same protocol, a second group of C57 mice were either enriched with IVA ($n = 12$) or experience the control condition ($n = 8$) for between 16 and 39 days after which EOGs were measured (Figure 8). For the turbinate II, Loc 1 recording site, IVA stimulus concentration was a significant factor as expected ($F_{2.22, 39} = 12.2$, $P < 0.0001$) as was enrichment condition ($F_{1, 18} = 13.9$, $P < 0.0015$) and the interaction between concentration and enrichment condition ($F_{3, 54} = 17.3$, $P < 0.0001$).

The same results were found for the turbinate III, Loc M recording site when IVA was the stimulus: concentration ($F_{1.62, 29.4} = 64.1$, $P < 0.0001$), enrichment condition ($F_{1, 18} = 35.8$, $P < 0.0001$) and their interaction ($F_{3, 54} = 8.1$, $P < 0.0002$) were all significant.

In our initial replication of Wang et al. (1993) we showed that the response to IAA was not different between the control and IVA-enriched group suggesting that induction was specific to some degree. Although IVA and IAA have similar chemical backbones, we wanted to further examine the specificity of induction by using another odorant with the same function group as IVA and chose OA, which, like IVA, is a fatty acid. For the turbinate II, Loc 1 recording site, OA concentration was a significant factor as expected ($F_{1.88, 33.8} = 76.2$, $P < 0.0001$), but enrichment with IVA did not affect the OA response ($F_{1, 18} = 1.0$, $P > 0.32$) and the interaction between OA concentration and enrichment condition was not significant ($F_{3, 54} = 0.20$, $P < 0.0001$). Similar results were found for the turbinate

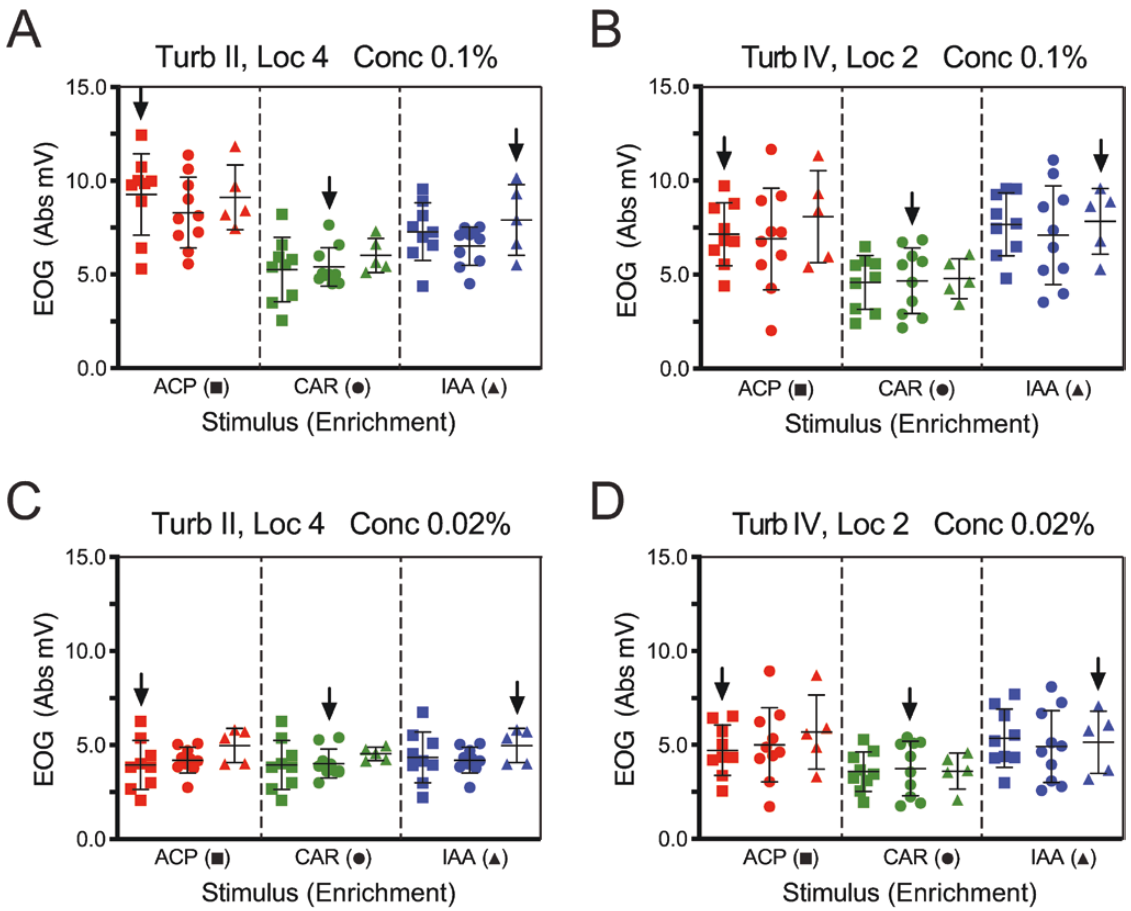

Figure 4. Absolute values of the EOG amplitudes in response to stimulation by ACP (red symbols), CAR (green symbols), or IAA (blue symbols) in mice enriched (duration 20 days) as adults with either ACP ($n = 9$), CAR ($n = 10$), or IAA ($n = 5$). Each of the 3 panels within a graph depict results from 1 stimulus odorant. For clarity, the enriched odorant for each panel is marked with a down pointing arrow. Large horizontal bars represent the mean; shorter horizontal bars represent the SEMs. (**A**) Results from turbinate III, Loc 4 using 0.1% v/v odorant concentrations. (**B**) Results from turbinate IV, Loc 2 using 0.1% v/v odorant concentrations. (**C**) Results from turbinate III, Loc 4 using 0.02% v/v odorant concentrations. (**D**) Results from turbinate IV, Loc 2 using 0.02% v/v odorant concentrations. None of the mean responses to the odorants used in enrichment are significantly different from the mean responses to the same odorant in animals enriched with one of the other 2 odorants.

III, Loc M recoding site when OA was the stimulus: concentration was a significant factor ($F_{1.68,\ 30.2} = 62.4$, $P < 0.0001$), but neither enrichment condition ($F_{1,\ 18} = 1.67$, $P > 0.2$) nor the interaction ($F_{3,\ 54} = 2.05$, $P > 0.12$) of concentration and enrichment condition were significant.

This experiment replicates in most details our initial demonstration of IVA induction (above) and adds to it further evidence for chemical specificity beyond functional group. Also, the replication of IVA induction allowed us to reexamine the relationship between enrichment duration and induction magnitude, but again we found no correlations (data not shown).

Induction recovery

In our previous studies of deprivation, mouse EOGs drop to normal magnitudes 2 weeks after removal of nasal plugs (Barber and Coppola 2015). In a similar effort to understand the dynamics of induction we enriched another group of C57 mice ($n = 10$) with IVA for 2 weeks and then removed the odorant for 2 weeks before measuring EOGs. Results were compared with controls for the first and second experiments described in the previous sections (Figure 9). For the turbinate II, Loc 1 recording location (Figure 1A), IVA stimulus concentration was a significant factor as expected ($F_{2.8,\ 71.1} = 132.1$, $P < 0.0001$) but enrichment condition (recover vs control 1 or control 2) was not ($F_{2,\ 25} = 1.5$, $P > 0.24$) nor was the interaction between concentration and enrichment condition ($F_{6,\ 75} = 0.64$, $P > 0.69$). Similar results were found for the turbinate III, Loc M recording location: IVA stimulus concentration was a significant factor ($F_{2.,\ 65.1} = 132.1$, $P < 0.0001$), but enrichment condition was not ($F_{2,\ 25} = 2.45$, $P > 0.1$) nor was the interaction between concentration and enrichment condition ($F_{6,\ 75} = 0.85$, $P > 0.50$). Thus, similar to the recovery after deprivation, IVA induction in C57 mice returns to baseline levels in 2 weeks at most.

Onset timing

Based on the experiments so far, it can be concluded that induction is fully manifest after at least 2 weeks of enrichment. To measure onset timing more precisely for comparison with deprivation, we exposed groups of C57 mice to 3 days ($n = 9$) or 7 days ($n = 9$) of IVA enrichment, whereas 3 mice that received sham enrichment (clean air) served as contemporaneous controls. We then measured EOG responses, as previously, in response to a log series of IVA and IAA (Figure 10). For the analysis of the IVA stimulus, we combined the new results with the pooled results of the above 2 experiments that used ≥14 day IVA enrichment. As expected, IVA stimulus

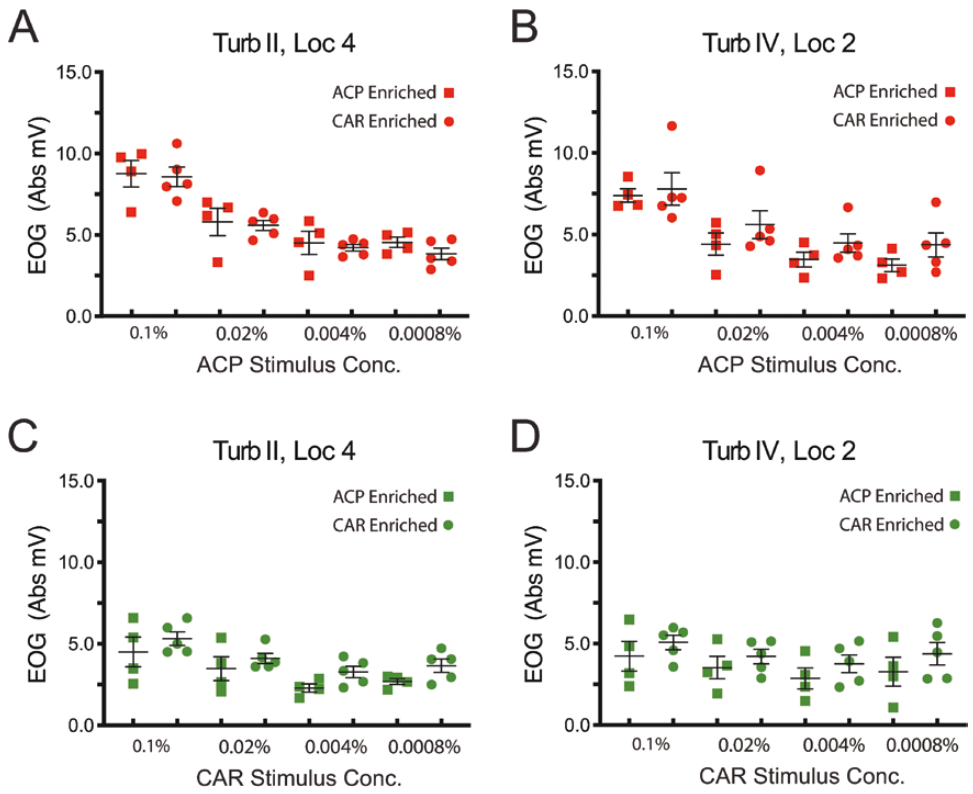

Figure 5. Absolute values of EOG amplitudes in response to stimulation by ACP (red symbols) or CAR (green symbols) in mice enriched from weaning (duration 55–56 days) with either ACP ($n = 4$) or CAR ($n = 5$). (**A**) Results from turbinate III, Loc 4 using 4 v/v concentration of ACP in mice enriched either with ACP (square) or CAR (circles). (**B**) Results from turbinate IV, Loc 2 using 4 v/v concentrations of ACP in mice enriched either with ACP (square) or CAR (circles). (**C**) Results from turbinate III, Loc 4 using 4 v/v concentration of CAR in mice enriched either with ACP (square) or CAR (circles). (**D**) Results from turbinate IV, Loc 2 using 4 v/v concentrations of CAR in mice enriched either with ACP (square) or CAR (circles). None of the ACP enriched versus CAR enriched comparisons within a concentration are significantly different.

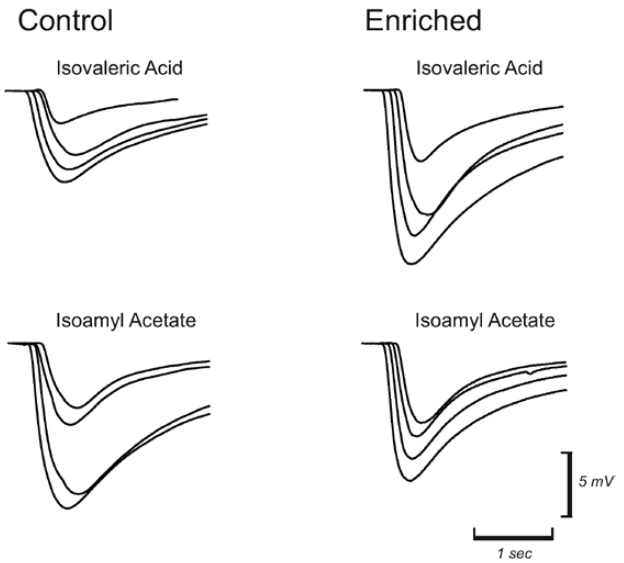

Figure 6. Representative EOG traces from C57 mice stimulated with log dilution series (10^{-2} to 10^{-5} M) of IVA (upper traces) IAA (lower traces). Left traces are from control mice. Right traces are from mice enriched daily with IVA for at least 2 weeks (see text). Note that larger amplitude EOGs at all concentrations for enriched mice responding to IVA, but not IAA.

concentration was a significant factor ($F_{2.1, 83.1} = 90.3$, $P < 0.0001$) for the turbinate II, Loc 1 recording site (Figure 10A), but so too was enrichment condition ($F_{3, 39} = 33.7$, $P < 0.0001$) and the interaction between concentration and enrichment ($F_{9, 117} = 6.6$, $P < 0.0001$). To test for differences between enrichment durations, a Tukey's multiple comparisons test was used to compare all means at each stimulus concentration separately. In summary, the results of these tests are consistent with the conclusions that 1) 3 days of enrichment did not cause a significant effect on EOG amplitude compared with controls; 2) 7 days of enrichment caused an increase in EOG magnitude at the highest 2 stimulus concentrations; 3) 7 days of enrichment was associated with significantly lower EOGs than ≥14 days of enrichment (pooled from experiments 1 and 2) at all stimulus concentrations however this last conclusion carries the qualification that there were slight difference between the enrichment methods between the ≥14 days group and the 3- and 7-day groups (see Materials and methods). By contrast, although IAA (Figure 10B) concentration, as usual, was a significant factor in the analysis ($F_{2.1, 37.2} = 118.1$, $P < 0.0001$), enrichment condition ($F_{2, 18} = 0.46$, $P > 0.64$) and the interaction between concentration and enrichment condition ($F_{6, 54} = 0.34$, $P > 0.91$) were not, showing yet again that induction is specific to the enrichment odorant.

For the turbinate III, Loc M recording site, the results were similar: IVA stimulus concentration ($F_{1.90, 73.3} = 63.1$, $P < 0.0001$); enrichment condition ($F_{3, 39} = 16.2$, $P < 0.0001$); and the interaction term ($F_{9, 117} = 3.8$, $P < 0.0003$) were all significant (Figure 10C). Tukey's test was used to examine the difference between enrichment durations for each stimulus concentration separately. The 3-day exposure group only had a significantly greater mean EOG amplitude than the control group for the 10^{-3} M concentration ($P < 0.02$). As for the 7-day exposure group mean, it was significantly greater than the control

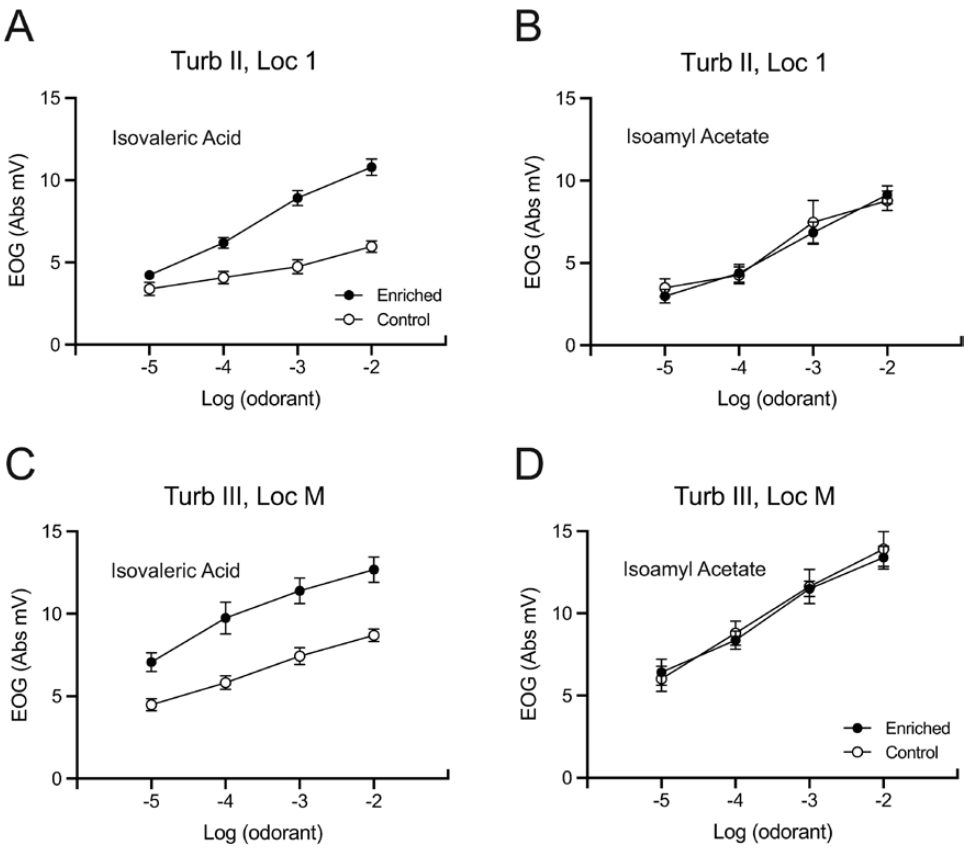

Figure 7. Mean (± SEM) EOG amplitudes (absolute values) from enriched (n = 10) or control (n = 10) C57 mice stimulated with a log dilution series (10^{-2} to 10^{-5} M) of IVA or IAA. (**A**) Results from turbinate II, Loc 1 when IVA was the stimulus. Values from enriched and control groups are significantly different (see text for details). (**B**) Results from turbinate II, Loc 1 when IAA was the stimulus. Values from enriched and control groups are not significantly different (see text for details). (**C**) Results from turbinate III, Loc M when IVA was the stimulus. Values from enriched and control groups are significantly different (see text for details). (**D**) Results from turbinate III, Loc M when IAA was the stimulus. Values from enriched and control groups are not significantly different (see text for details).

group mean for all but the lowest stimulus concentration and it was significantly less than the ≥14-day enrichment group (pooled from experiments 1 and 2) mean only for 10^{-3} M concentration ($P < 0.03$). As above, IAA (Figure 10D) concentration was a significant factor in the analysis ($F_{1.8, 33.1} = 113.9$, $P < 0.0001$), but enrichment condition ($F_{1, 18} = 2.20$, $P > 0.14$) and the interaction between concentration and enrichment condition ($F_{6, 54} = 1.50$, $P > 0.19$) were not. These results suggest that IVA enrichment causes a significant increase in the mean EOG response to this odorant after 7 days of enrichment, an effect that increases through 14 days of enrichment.

Strain specificity

In the experiment described thus far, our inability to show induction using ACP, CAR, or IAA in CD-1 mice stands in sharp contrasts with our repeated demonstrations, consistent with Wang et al. (1993), of induction in C57 mice using IVA as the enrichment odorant. To test the possibility that inducibility might be a peculiarity of certain odorants rather than strain specificity, we tested CD-1 mice induction with IVA (Figure 11). This also allowed us to rule out an effect of the differences in enrichment method between the first CD-1 mouse experiments (those involving ACP, CAR, or IAA) and the subsequent C57 mouse experiments the latter of which followed the protocol by Wang et al. (1993). The following analysis compares the induction results for the CD-1 strain with the pooled data from both C57 strain experiments, using exactly the same enrichment method (see Materials and methods).

For turbinate II, Loc 1, IVA stimulus concentration was a significant factor in explaining mean EOG amplitude ($F_{2.1, 115.9} = 68$, $P < 0.0001$); enrichment condition ($F_{3, 56} = 21.3$, $P < 0.0001$) and the interaction term ($F_{9, 168} = 15.9$, $P < 0.0001$) were also significant (Figure 11C). Tukey's test was used to examine the difference in induction between strains for each stimulus concentration separately. To summarize the numerous Tukey comparisons, the tests are consistent with the conclusions that 1) CD-1 enriched mice are not different than CD-1 controls for any stimulus concentration; 2) CD-1 and C57 controls are only significantly different at the highest stimulus concentration; 3) C57-enriched EOG means are significantly greater than CD-1-enriched means at the highest 2 stimulus concentrations. When IAA was the stimulus (Figure 12B), concentration, as usual, was a significant factor in the analysis of EOG means ($F_{1.9, 34.9} = 135.4$, $P < 0.0001$), but enrichment condition ($F_{1, 18} = 1.01$, $P > 0.91$) and the interaction between concentration and enrichment condition ($F_{3, 54} = 0.63$, $P > 0.60$) were not, establishing, again, that IVA enrichment does not influence the mean IAA response at any concentration. As above, the C57 mouse data for the IAA stimulus were excluded in Figure 12B for clarity.

For turbinate III, Loc M (Figure 12C), IVA stimulus concentration was a significant factor in explaining mean EOG amplitude ($F_{2.2, 101.6} = 146.3$, $P < 0.001$) as was enrichment condition ($F_{3, 46} = 18.0$, $P < 0.0001$), and the interaction term ($F_{9, 138} = 3.3$, $P < 0.0001$). Again, Tukey's was the post hoc test of choice. To summarize, the results are consistent with the conclusions that

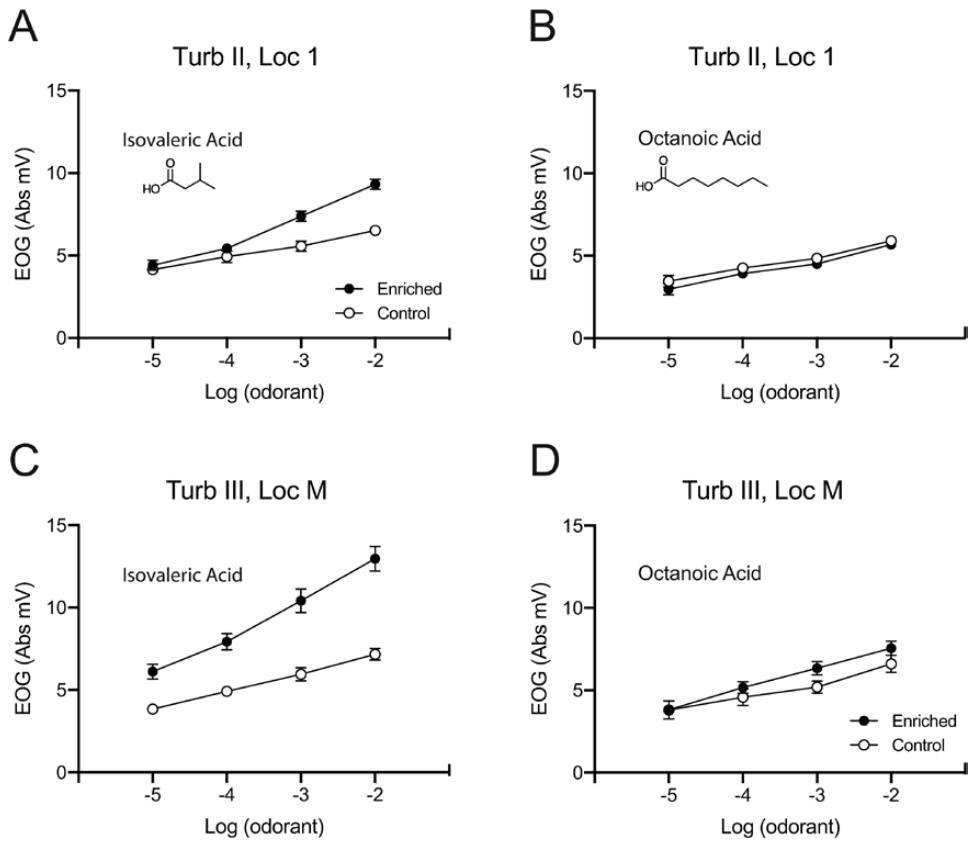

Figure 8. Mean (± SEM) absolute values of EOG amplitudes from enriched (n = 11) or control (n = 8) C57 mice stimulated with a log dilution series (10^{-2} to 10^{-5} M) of IVA or OA. (**A**) Results from turbinate II, Loc 1 when IVA was the stimulus. Values from enriched and control groups are significantly different (see text for details). (**B**) Results from turbinate II, Loc 1 when OA was the stimulus. Values from enriched and control groups are not significantly different (see text for details). (**C**) Results from turbinate III, Loc M when IVA was the stimulus. Values from enriched and control groups are significantly different (see text for details). (**D**) Results from turbinate III, Loc M when OA was the stimulus. Values from enriched and control groups are not significantly different (see text for details).

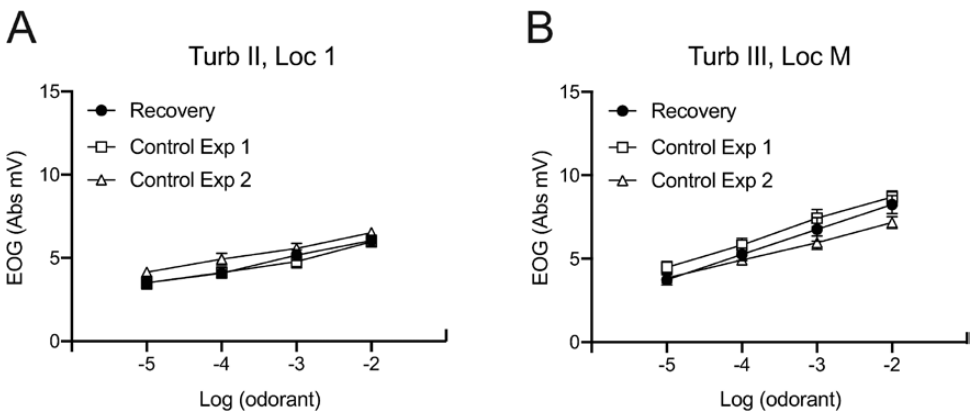

Figure 9. Mean (± SEM) absolute values of EOG amplitudes from C57 mice (n = 10) stimulated with a log dilution series (10^{-2} to 10^{-5} M) of IVA. The recovery group was enriched with IVA for 2 weeks after which IVA was removed for 2 weeks before EOGs were recorded. Control groups are replotted from Figure 7 (Exp. 1) and Figure 8 (Exp. 2). (**A**) Data from turbinate II, Loc 1. The 3 groups are statistically indistinguishable (see text for details). (**B**) Data from turbinate III, Loc M. The 3 groups are statistically indistinguishable (see text for details).

1) mean EOG amplitude of enriched CD-1 mice is not different from that of CD-1 controls for any IVA concentration; 2) mean EOG amplitude for C57 controls is significantly lower than that for CD-1 mice at all stimulus concentration; 3) C57 enriched EOG means are not significantly different from CD-1 enriched means at any stimulus concentrations. When IAA was the stimulus (Figure 12D), concentration was a significant factor in the analysis of EOG means ($F_{2.3, 42.2.9} = 178.3$, $P < 0.0001$), but enrichment condition ($F_{1, 18} = 2.2$, $P > 0.16$) and the interaction between concentration and enrichment condition ($F_{3, 54} = 1.3$, $P > 0.27$) were not. C57 data were not included in Figure 12D for clarity.

Taken together, the comparison of induction in CD-1 and C57 mice support the interpretation that IVA enrichment has no effect, in the former strain, on EOG amplitudes in response to IVA or IAA

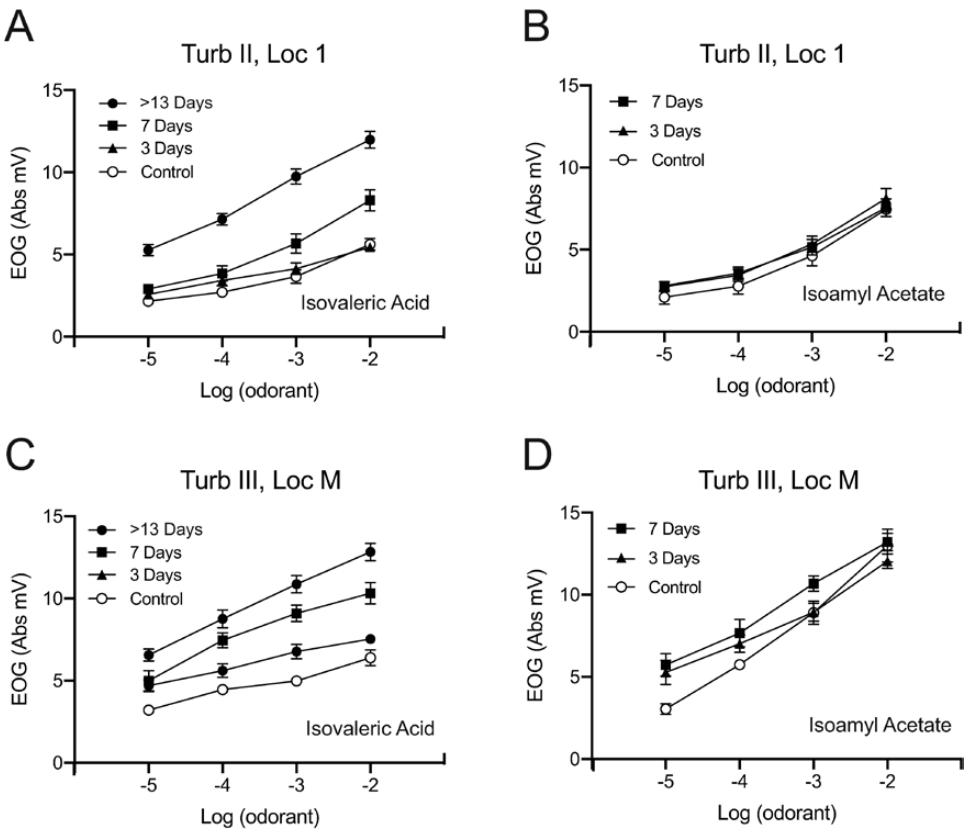

Figure 10. Mean (± SEM) absolute values of EOG amplitudes from IVA-enriched or control C57 mice stimulated with a log dilution series (10^{-2} to 10^{-5} M) of IVA or IAA. Enrichment durations were 0 days (control; $n = 3$), 3 days ($n = 9$), or 7 days ($n = 9$). The >13-day enrichment data ($n = 21$) were pooled from Figures 7 and 9. (**A**) Results from turbinate II, Loc 1 when IVA was the stimulus. Values of enriched groups are significantly different from the control group at 7 days (see text for details). (**B**) Results from turbinate II, Loc 1 when IAA was the stimulus. Values from enriched and control groups are not significantly different (see text for details). (**C**) Results from turbinate III, Loc M when IVA was the stimulus. Values from enriched groups are significantly different from control group at 7 days (see text for details). (**D**) Results from turbinate III, Loc M when IAA was the stimulus. Values from enriched and control groups are not significantly different (see text for details).

at any of the concentrations tested. Although there is some evidence from the more dorsal recording location that IVA induction in C57 produces mean EOG responses that are greater than those in CD-1s, the majority of the data suggest that induction is a process whereby subnormal IVA responses in control C57 are brought up to the range displayed by CD-1 outbred mice which we assume to be the normosmic mouse response.

Discussion

Though stimulus deprivation and enrichment have long been useful tools in olfactory research, their effects have seldom been compared in the same study. For UNO deprivation, we extended the results of our previous work by showing that compensatory responses to deprivation are fully manifest in adult mice 7 days after naris plugs are inserted (Barber and Coppola 2015). We also show, for the first time, that UNO is not detectably detrimental to the gross anatomical appearance or EOG responses of either the ipsilateral or contralateral OE, even after year-long survival periods. For enrichment in CD-1 mice, we were unable to detect any effect of either late-onset, short-duration or early-onset, long-duration odorant enrichment for any of 3 test odorants. However, we were able to replicate a previous study showing that C57 mice, congenitally hyposmic for IVA, have EOG responses enhanced by exposure to that odorant. We also added new findings: First, we extend our knowledge of effect specificity by showing that responses to even a close homologue of IVA, OA, is not induced by IVA enrichment; second, we show that induction is statistically significant by 7 days after enrichment and at its maximum by 14 days; third, complete recovery (return to control levels) occurs by 2 weeks (at most) after ceasing odorant enrichment; fourth, as was the case for other odorants, CD-1 mouse EOG responses are not affected by an *identical* IVA enrichment regimen that induced C57 mice.

When it was first discovered in a heroic set of behavioral genetic studies, the hyposmia of C57 mice for IVA must have seemed like a breakthrough (Wysocki et al. 1977). Amoore (1967), inspired by Guillot's (1948) previous speculation, believed that specific anosmias were the key to discovering odor primaries which were, he thought, the "Rosetta Stone" of the olfactory code. Here was a mouse specific anosmia model with all its appertaining experimental advantages, first among them being the possibility of genetic manipulation. More intriguing still was the discovery in 1989 of androstenone induction in humans followed a few years later by the demonstration of androstenone and IVA induction in mice (Wysocki et al. 1989; Wang et al. 1993). Although the intervening years have not been kind to Amoore's (and his acolyte's) program, specific anosmias and odor induction remain absorbing mysteries.

Human-specific anosmia and induction

Originally thought to be rare, studies over the last 50 years have established that specific anosmia or at least specific hyposmia in

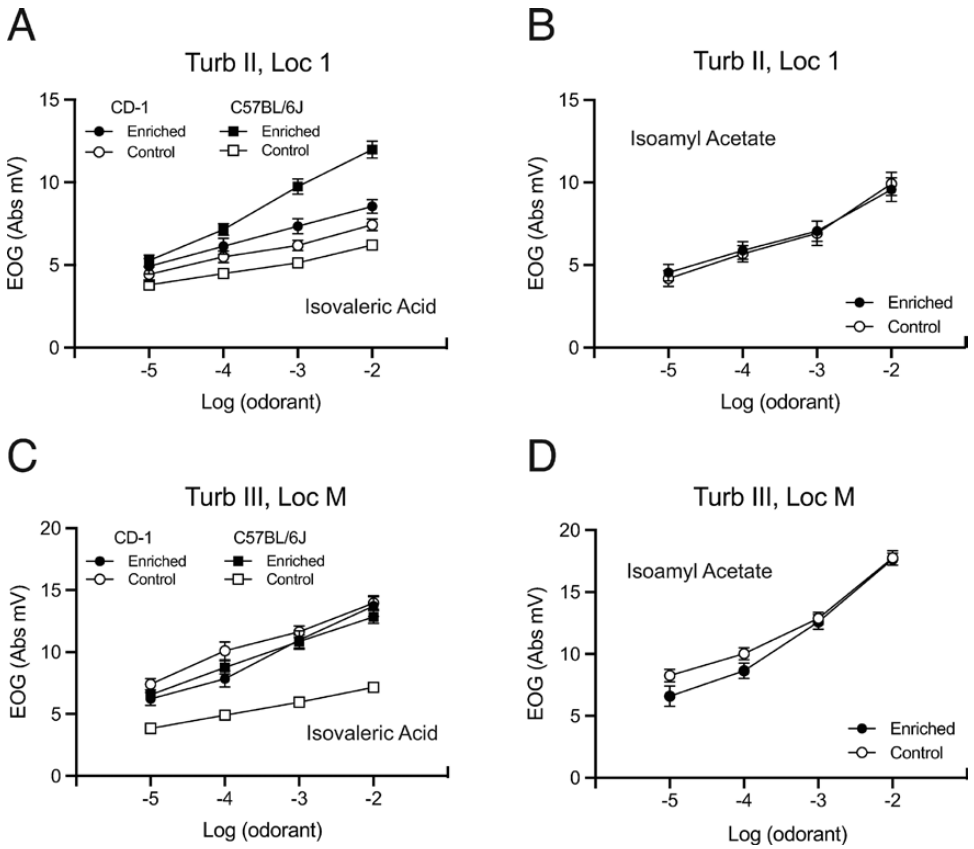

Figure 11. Mean (± SEM) absolute values of EOG amplitudes from enriched or control CD-1 ($n = 20$) and C57 mice ($n = 22$) stimulated with a log dilution series (10^{-2} to 10^{-5} M) of IVA or IAA. Data for C57 mice are pooled from Figures 7 and 8 for comparison. (**A**) Results from turbinate II, Loc 1 when IVA was the stimulus. Values from enriched and control CD-1 mouse groups are not significantly different (see text for details). (**B**) Results from turbinate II, Loc 1 when IAA was the stimulus. Values from enriched and control groups are not significantly different (see text for details). (**C**) Results from turbinate III, Loc M when IVA was the stimulus. Values from enriched and control CD-1 mouse groups are not significantly different (see text for details). (**D**) Results from turbinate III, Loc M when IAA was the stimulus. Values from enriched and control groups are not significantly different (see text for details).

humans may be the rule rather than the exception; that is, if tested with enough odorants, virtually everybody, likely, would be found to be anosmic to one or more odorants that most of the rest of the population can smell (Amoore 1967; Triller et al. 2008; Croy et al. 2015). As noted above, the enigma of anosmia is multiplied by the companion phenomenon of induction. Although most studies in humans have targeted androstenone induction (Van Toller et al. 1983: Wysocki et al. 1989; Wang et al. 2003; Mörlein et al. 2013), evidence of induction to dozens of other compounds has emerged (Dalton et al. 2002; Cain and Schmidt 2002; Croy et al. 2015). Despite many advances, the mechanism of induction remains unknown. The original (and still most common) proposal that induction is due to changes in the olfactory sensory neuron (OSN) population has little direct support despite the decades that have elapsed because the phenomenon was discovered.

Considering what is known about human olfactory induction, it seems possible that it is only superficially similar to the induction-like phenomenon in mice. Justifying this point of view are the facts that human induction requires only a few seconds per day of odorant sniffing while mouse induction involves 16 h/day of immersive odorant exposure. In the few cases where we have data, the genetic basis of a particular specific anosmia in the mouse is different than that in humans. Take IVA anosmia, for example, it has a multilocus basis in C57 mice that possibly includes both receptor and general olfactory-sensitivity-related genes, while the human genetic picture for IVA sensitivity is completely different (Griff and Reed 1995; Zhang and Firestein 2002; Menashe et al. 2007). Indeed, specific anosmia/hyposmia in nonhuman animals appears to be a peculiarity of a small number of inbred mouse strains as it is apparently unknown in outbred or wild-derived rodents. Finally, although C57 mice may well be hyposmic to IVA, the evidence that they or any other nonhuman animals are anosmic to standard odorants is unconvincing (Pourtier and Sicard 1990). Indeed, we measured robust, larger-than-blank responses in C57 mice to all concentrations of IVA. Whatever the merits of our surmise the following discussion assumes that odorant induction in human-specific anosmics has a different cause than the C57 mouse/IVA induction model.

Mouse induction

Despite its original promise, the C57 mouse/IVA odorant anosmia model has not shed much light on specific anosmia or odor induction. Indeed, to our knowledge, the genetic basis of this olfactory deficit remains obscure and the induction component lacks a viable mechanism (Griff and Reed 1995; Zhang and Firestein 2002). However, we have already referred to an established induction-like process in the mouse OE, compensatory plasticity (Coppola 2012). Perinatal UNO leads to increases in transductory pathway proteins in OSNs from the deprived OE including adenylyl cyclase (Waguespack et al. 2005; Coppola et al. 2006). Microarray analysis of the transcriptomic changes in the deprived OE after UNO,

likewise, shows significant upregulation of transcripts for adenylyl cyclase, the olfactory cyclic nucleotide-gated channel and the olfactory G-protein, and numerous olfactory receptors (ORs) (Coppola and Waggener 2012). And, as previously noted, deprived-side increases in the response magnitude of the EOG are found following perinatal or adult UNO (Waggener and Coppola 2007; Barber and Coppola 2015). Finally, mice that received perinatal UNO and were limited to the use of their deprived-side olfactory system as adults showed enhanced behavioral sensitivity to odorants (Angely and Coppola 2010). The mechanism of this compensatory process is only a matter of speculation at this time, but we presuppose the basic elements of any other homeostatic process: a set point, error signal, and negative-feedback mechanism—hardly a radical proposition for a sensory neuron (Davis 2006; Lee and Fields 2020). An experimental program to identify the mechanism of olfactory compensatory plasticity may have to await a more solid understanding of OSN adaptation and desensitization (Zufall and Leinders-Zufall 2000; Kato et al. 2014). However, the adaptive significance (in the evolutionary sense) of such a compensatory process seems clear: to normalize the dynamic range of OSNs to a variable olfactory milieu (Fain et al. 2001).

Though superficially counterintuitive, we propose, based on our findings, that IVA induction in C57 mice has the same underlying mechanism as UNO-triggered compensatory plasticity. And the same mechanism may apply to other forms of induction brought on by chronic odorant enrichment. More specifically, we posit that chronic exposure to high concentrations of purified odorants, through the process of adaptation and receptor desensitization, eventually causes a compensatory rebound of the olfactory-transductory pathway after the enrichment odorant is removed. As in compensatory plasticity, we envision that components of the transductor pathway like adenylyl cyclase, cyclic-nucleotide-gated channels, and ORs would be upregulated in reaction to the activity reduction brought on by unrelenting adaptation. The existence of such a process is supported by the similar dynamics of UNO compensation and odorant induction. First, we have shown that both are manifest after approximately 7 days of onset. Second, we have shown that both disappear after 2 weeks of treatment removal, either naris plug or chronic odorant. Third, we have shown that both treatments result in similar magnitudes of EOG enhancement. Fourth, deprivation and enrichment have been shown in other studies to cause similar effects at the transcript level causing up regulation of some ORs and downregulation of others (Coppola and Waggener 2012; Ibarra-Soria et al. 2017). If this hypothesis is correct, at least 2 factors need to be accounted for: the specificity of the effect in C57 inbred mice and the lack of effect in CD-1 outbred mice. To account for the former, we posit that C57 mice may lack IVA receptors resistant to chronic adaptation—perhaps these are high affinity receptors for IVA (Zhang and Firestein 2002). If true, it follows that chronic adaptation might lead to a compensatory rebound in these receptors once odorant enrichment is ended. Concerning the latter issue, we propose that CD-1 mice could not be induced because they possess a preponderance of adaptation-resistant and low-affinity receptors that experience only a small or nonexistent rebound once odorant enrichment ends, though clearly other explanations are possible. It is interesting in this regard that different mouse ORs are known to have markedly different basal activity levels (Reisert 2010).

Conclusions

Based on key differences, it is proposed that olfactory induction in humans may be a different process from the olfactory induction observed in some inbred mouse strains despite the superficial similarity of these phenomena. Conversely, based on similar dynamics, it is proposed that induction in mice may have the same underlying cause as compensatory plasticity, the well-established heightened sensitivity of OSNs after a period of odorant deprivation. It is further proposed that both phenomena are controlled by homeostatic processes of OSNs, whose precise explication will await a clearer understanding of olfactory peripheral adaptation and receptor desensitization. From this perspective, induction, at least in animals, does not appear to represent a form of perceptual learning, as has been oft suggested, but is rather a side effect of the adaptation process. Thus, deprivation and enrichment—these seeming opposites—may have identical effects on the olfactory periphery because they ultimately engage the same mechanism for normalizing sensory activity in the face of environmental change.

Funding

This work was supported by grant IOS-1655113 to DMC from the National Science Foundation (USA).

Acknowledgments

The authors acknowledge the technical assistance of Alyson Blount and literature collection assistance of Kelli Salmon.

Conflict of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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