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Large-Scale Investigation of the Olfactory Receptor Space Using a Microfluidic Microwell Array

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Abstract

The mammalian olfactory system is able to discriminate among tens of thousands of odorant molecules. In mice, each odorant is sensed by a small subset of the approximately 1,000 odorant receptor (OR) types, with one OR gene expressed by each olfactory sensory neuron (OSN). However, the sum of the large repertoire of OR/OSN types and difficulties with heterologous expression have made it almost impossible to analyze odorant responsiveness across all OR/OSN types. We have developed a microfluidic approach that allowed us to screen over 20,000 single cells at once in microwells. By using calcium imaging, we were able to detect and analyze odorant responses of about 2,900 OSNs simultaneously. Importantly, this technique allows for both the detection of rare responding OSNs as well as the identification of OSN populations broadly responsive to odorants of unrelated structures. This technique is generally applicable for screening large numbers of single cells and should help to characterize rare cell behaviors in fields such as toxicology, pharmacology, and cancer research.

INTRODUCTION

In the nose, olfactory sensory neurons (OSNs) translate odorant chemical signals into unique neuronal signals that guide many important behaviors. Odors have been shown to help identify and categorize food [1], provide associative signals for memory storage and retrieval [2–7], and strongly influence sexual [8, 9], parental [10–13], and social behaviors [14–16]. Odorants activate odorant receptors (ORs), expressed in the membrane of the OSNs of the main olfactory epithelium (MOE) [17, 18]. In mice, there are around 1,000 types of ORs, and any given OSN only expresses one type of OR [19], to which different odorants can bind with different affinities. Thus, any odorant (at a given concentration) can be thought of as being encoded by the brain as a set of combinatorial affinities between itself and each of the ORs [20]. To date, although extensive searches for matches between ORs and odorants have yielded great insight into the olfactory code [21–23], the number of matches between ORs and odorant ligands is relatively few. One reason is that given the ~1,000 OR/OSN types, no available technique allows for probing all the ORs at once with multiple odorants. Calcium imaging of glomerular activation in the olfactory bulb has demonstrated a spatial activation map (with one glomerulus corresponding to one OR type) [24–27]). However, only the dorsal surface of the OB can be imaged by this approach. Calcium imaging of intact MOE fragments only covers a small portion of the whole MOE

area [28]. A common strategy to probe OR activation is to use dissociated OSNs in acute cultures [29]. However, since the cells have to be seeded at low density to avoid clumping, the number of ORs represented in any given image is typically very low such that covering the whole OR space requires at least dozens of experiments. Finally, ligand identification of ORs by heterologous expression has proven difficult due to problems with functional expression of ORs; ligands have been identified for only a small fraction of ORs [30–32].

The advent of soft lithography [33] has allowed for the development of inexpensive microfluidic methods to assay single cells at high throughput [34–38]. Here we describe a large-scale imaging and microfluidic platform for simultaneously detecting the odorant-evoked calcium responses of up to several thousand dissociated OSNs in a background of approximately 28,000 dissociated cells. Therefore, given approximately 1,000 ORs [19], the majority of the OR space can be considered to be statistically represented in any given image. Using this approach, in one experimental run we could compare responses to four different fruity/floral smells (vanilla, rose, berry, and banana) by approximately 2,900 OSNs. We found that while all four odorants activated similar numbers of OSNs, the second-largest group of OSNs was narrowly tuned to detect banana only. Surprisingly, the largest group of OSNs was broadly tuned to respond to all four odorants.

RESULTS

Thousands of dissociated olfactory sensory neurons in a microwell array of a microfluidic chamber

Placing dissociated cells in a microarray allows for high-density imaging of individual cells [39]. We have incorporated a microwell array, similar to that which we used previously for cells in static culture conditions [39], into a microfluidic chamber. The wells have the ideal size to trap single dissociated OSNs (20 μm -diameter, 10 μm -depth) (Fig. 1A). Using 4x magnification, we could visualize ~32,000 wells simultaneously (~1,560 wells/ mm^2). The microfluidic system consists of a single, central “wash” channel for saline and 8 stimulant channels for different odorants on the side (Fig. 1B). Cells were loaded into the device via the central line and allowed to settle (1–3 cells per well). The lines were then flushed with fluid, taking away excess cells and leaving ~70–90% of the wells filled. Individual cells remained trapped in the wells (Fig. 1C, inset), where they were protected from shear stresses by the laminar flow conditions above the wells. Control experiments with calcein-AM and ethidium homodimer [40] established that ~95% of the cells in the device were viable, yielding approximately ~28,000 cells per image.

The timeline of a typical perfusion experiment is shown schematically in Fig. 2A. Control experiments with fluorescein underscore the advantages of using microfluidic application as compared to bulk fluid flow (Fig. 2B). The lack of turbulence (laminar flow) at this scale allows relatively quick fluid exchange without dead volumes in the microwells chamber. For these fluorescein measurements, images were taken once per second. Despite the very simple application technology used here (gravity perfusion and pinch valves), time to fluorescence saturation was ~2 sec, and time to washout was ~4–6 sec. This rapid fluid exchange and the small fluid volumes enable fast and reproducible odorant application and removal.

Large-scale imaging and analysis of calcium responses

Analysis of such a large number of cells (~30,000) at once requires first, the development of appropriate methods to automate the identification of responding cells and second, the criteria to efficiently to characterize their response profiles. As in prior studies [20], we identified OSNs on the basis of their calcium response to a control pulse of KCl (85 mM).

The increases in intracellular calcium were detected using fluo4-AM, a non-ratiometric calcium indicator whose fluorescence increases with elevations in intracellular calcium. Odorant and control fluid applications were in general done as double pulses of 40 sec each with a 120 sec rinse in between to allow for recovery. KCl (in saline) was delivered in two consecutive pulses of 8 and 12 sec of duration; the difference between the first and second KCl stimulation was to ensure that there was activation of the cell at the second exposure. Images were taken every 4 seconds (200 ms exposures) using a 4x objective, yielding approximately 9 pixels per cell (~25 pixels per well).

Responding OSNs were identified as follows. Although the cells were in microwells, the registration and location of the cell within the well was too variable to predict their location. Given the extremely large number of potential cells, we created a software algorithm in Matlab to allow semi-automated identification of cells (Fig. 2C,D,E). First, thirty cells with an obvious calcium response to KCl were selected by hand (example in Fig. 2C). The average response of these strongly-responding cells (Fig. 2D, red trace) was used as a “template” to identify responsive cells as follows. At each time point, each pixel in the image was given a score (ranging from 0 to 255) for the correlation of the pixel’s fluorescence intensity compared to that of the template. A grayscale map over the full field of view was created using the pixel’s score, with higher responses appearing brighter (Fig. 2E, left image). Assuming that individual cells must be represented by at least 9 contiguous pixels (approximate diameter of an OSN), we thresholded the images to display clusters of responding pixels (Fig. 2E, middle image). Each putative cell was visually confirmed by a Matlab movie (Suppl. Fig. 1) that displays all of the responsive cells in a virtual array (Fig. 2E, right). Approximately 2% of the cells moved during stimulus application and were removed from further analysis as potential false positives, and almost all pixel clusters were confirmed as cells (~99%). With this approach, out of the ~28,000 cells imaged we identified over 1,000 KCl-responsive cells in each of two separate experiments (1359 and 2929 cells), the larger of which is presented in detail in this paper.

Lastly, responsiveness to individual odorants was determined as follows. As shown schematically in Fig. 2A, cells were exposed first to control solutions (e.g. DMSO vehicle), then to odorants, with a final exposure to KCl to confirm viability throughout the experiment. Calcium responses of individual cells were determined using Metamorph (Molecular Devices) over large regions (20 × 20 pixel boxes) centered on the cell locations determined as above with Matlab. The change in fluorescence intensity versus the initial baseline intensity for each new odorant ($\Delta F/F$) was followed for each cell. KCl-positive cells were considered odorant-responsive if they met the following conditions: at least one response to an odorant and to KCl with a $\Delta F/F \geq 2\%$, and a DMSO response with a $\Delta F/F \leq 1\%$. Although individual cells displayed a wide range of response strengths ($\Delta F/F$ from 2 to ~20%), we chose to simplify the analysis by rating a cell as either responsive or not responsive to a particular odorant.

Odorant responses

The cells were sequentially exposed to four fruity or floral smelling odorants with varied structures: vanillin (4-Hydroxy-3-methoxybenzaldehyde, vanilla smell), geraniol (roselike, 3,7-Dimethyl-2,6-octadien-1-ol), ethyl butyrate (berry, ethyl butanoate), and benzyl acetate (jasmine or banana candy). Out of the 28,000 imaged cells, 2929 cells (~10%) were presumptive OSNs (KCl-responsive). Out of the KCl-responsive cells, 422 cells (14%) responded to an odorant and/or the control solution of DMSO (0.02% final concentration). Although DMSO itself has a smell, the DMSO-responsive cells (276 cells) were considered to be false positives as the odorants were dissolved in the same concentration of DMSO. Therefore, of ~3,000 OSNs imaged, 146 cells were considered responsive to at least one of the four tested odorants. This represents only 5% of the OSNs (~0.5% of the total number of

imaged cells). The number of cells responding to a given odorant (i.e. responding, *at least*, to that odorant) was similarly low for all the odorants (range: 81–106), or 2.7–3.6% of the OSNs.

Two representative cells per group, the lowest and highest responder of each group (except for ethyl butyrate which had only one responder), are shown in Fig. 3. For any given response profile, the number of cells that respond with a similar profile is shown in the right column of Fig. 3. A similar representative sampling of the DMSO-positive cells is shown in Suppl. Fig. 2 for comparison. In general, odorant responses were much lower in magnitude than the KCl responses.

Identification of OSNs that display rare response patterns

Interestingly, due to the large number of OSNs imaged at once using this technique, we were able to identify OSNs with rare response patterns. These are divided in two classes, the first being OSNs activated by selective odorants and the second being OSNs activated by particular combinations of odorants.

The distribution of OSN responses to the odorants is summarized in Fig. 4. Individual cells varied in the specificity of their responses to odorants. Cells that responded to all four odorants made up the largest group of odorant-responding OSNs, comprising 25% of the odorant-responding population (37 of 146 cells). In fact, cells that were broadly tuned (responding to 3 or 4 odorants) made up 47% of the responsive cells. Likely due to the high percentage of broadly tuned cells, the number of cells responding to a given odorant (i.e. responding to that odorant *at least*) was similarly high for all of the odorants (55–73%). In contrast, cells that responded to a single odorant only were less common (less than 1% to 5.5% of odorant-responsive cells), with the exception of the benzyl acetate group (17%). Note that these numbers of responding cells represent only 0.03 to 0.9% of OSNs. Three of the four odorants share a similar distribution of fewer narrowly tuned cells, rising to more broadly tuned cells. However, for one odorant, benzyl acetate, the number of cells narrowly tuned to only benzyl acetate is relatively high, so that it is roughly equivalent to the number of cells broadly tuned to benzyl acetate and other odorants. Importantly, all types of odorant-responsive groups identified are relatively rare, given that the largest group (37 cells) only represents 1.3% of the total number of OSNs (themselves about 10% of our screened cells). This fact underscores the advantage of being able to screen large numbers of OSNs to find multiple examples for the relatively common OSN types, and to be able to isolate some of the more rare types at all.

DISCUSSION

Here we describe a microfluidic approach that enables us to examine virtually the entire OR repertoire in a single experiment. In our device, we record simultaneously from ~3,000 presumptive OSNs. Assuming each imaged cell randomly chooses to express 1 of the ~1,000 odorant receptors, we have on average 3 of each OR type in our experiment. Strictly speaking, the entire OSN population and the entire range of ORs will have equal statistical representation inside the device only if all the OSNs expressing the same OR are homogeneously distributed throughout the MOE (or one of its zones), an assumption that cannot be presently tested but that we use as a working hypothesis. We emphasize that in previous work from other labs, the acquisition of a sufficient data set required painstaking repetition of experiments whereas we could obtain them in a single experiment.

Here we analyze in detail responses from 2929 OSNs imaged at once, exploring the OR/OSN repertoire for some fruity or floral odorants. We found that a similar percentage of OSNs responded to each of the four odorants (2.6–3.6% of the KCl-positive cells). Note that

for simplicity, in the present study we do not vary some known important parameters for OSN responsiveness, such as odorant concentration and stimulus order which may affect adaptation. Despite the relatively high concentration of our ligands, the percentages of responsive OSNs are smaller than that seen for other structural types of ligands in some other studies, such ~8.5% for carvones in one large study [41], and 6 to 21% for an aldehyde (depending on concentration) [42].

About 26% of the odorant-responsive OSNs were “narrowly tuned” to one of the 4 odorants of various structures (including alcohol, ether, ester, and benzyl groups). Looking at aldehydes, Araneda et al. found that 24% of responding cells responded to only one of 9 aldehydes at 300 μ M [42]. Interestingly, although a similar percentage of OSNs responded to each odorant in our study, a relatively large number of odorant-responsive OSNs responded to benzyl acetate only (17% for benzyl acetate, 0.7–5.5% for others). With more exposures to a larger diversity of odorants, it should be possible to discern whether the ~17% of cells responding to benzyl acetate form a unique subgroup of narrow tunability, or whether they are a heterogeneous group of cells expressing ORs with various response profiles.

A large percentage (25%) of the odorant-responsive cells detected all 4 odorants. Using a mixture of a large number of odorants would reveal whether the cells that respond to all of the odorants used here are even more broadly responsive to a large number of structurally-unrelated odorants. Our findings are consistent with other overlaps reported in the literature. For example, Bozza et al. [43] showed that a single cell type can respond to five out of six mixtures of eight odorants. Araneda et al. examined the responses of dissociated rat OSNs to several aldehydes [42] and found that 1 or 3% of the cells responded to all 9 aldehydes (at 30 or 300 μ M, respectively). A recent study looking at heterologously expressed ORs and a panel of 93 odorants identified ORs that were broadly tuned to odorants of widely varied structure [31].

Since in the mouse there are ~1,000 ORs and we obtain 2,929 KCl-responsive cells, our device should contain on average 3 cells for each OR assuming that a) all the KCl-responsive cells are OR-expressing OSNs; b) all the OE areas are sampled equally well by the surgical and isolation procedure; and c) each OR is equally represented in the mouse OR space (some ORs are expressed in fewer cells and some are more highly expressed [44]). By this logic, the set of 37 cells responding to all 4 odorants should contain ~12 different OR types, nearly 1% of the OR repertoire. We find it surprising that 1% of the OR repertoire is able to respond to all four of a group of randomly selected fruity or floral odorants, some of which have no structural features in common. There are several possible explanations that can be hypothesized for this puzzling observation. 1) There is an OR that is highly over-represented in the nose and that happens to be tuned exactly to our set of four odorants (an unlikely scenario). 2) There is odorant-independent activation, for example a mechanosensitive-OSN response to changes in flow [45] (unlikely as the cells didn't respond to a pulse of DMSO). 3) There is a subset of ORs that is broadly tuned (if at all), in which case broadening our panel of odorants should clarify the specificity of the OSNs. Such broadly-tuned cells could potentially widen the capacity of the olfactory system to detect diverse chemical structures; the loss in specificity may be offset by the large-scale combinatorial nature of OR coding. Future experiments may explore the latter possibility.

In these experiments we only examined responses to fruity/floral odorants. In future experiments with other classes of odorants we could ask the following questions. Do other odorant classes with very different perceptual qualities show similar distributions of OSN responsiveness? Do some types of odorants only activate narrowly tuned OSNs? How about odorants, such as thiols, that are very unpleasant at very low concentrations? Are there very

few, very specific, high affinity receptors? Do these compounds also activate other, less specific receptors as well?

Interestingly, some of our KCl positive cells may represent recently described olfactory epithelial cells of unknown function that respond to odorants and to KCl, but do not express components of the canonical cAMP pathway and rather appear to utilize a PLC pathway [46]. These cells are present at about 5% of the number of mature OSNs, but, in contrast, they do not connect to the olfactory bulb nor express olfactory marker protein. In that study, a large percentage of these cells (80%) responded to a mix that contained one of our odorants, ethyl vanillin, though only 1 of 7 cells responded to a mix of 4 other odors. In future experiments, these cells could be distinguished from OSNs by application of the phosphodiesterase inhibitor, IBMX, which selectively stimulates OSNs [46].

In conclusion, we have demonstrated a proof-of-principle, high-throughput OSN screening system that covers the majority of the OR space, allowing the identification of OSNs with rare response patterns. This system traps and maintains isolated OSNs in place for odorant exposure, and can be easily scaled-up and automated with on-chip microvalves [47] that control the fluid flow into the device. The ability to scan through cocktails of odorants and establish an odorant molecule profile for each cell while probing the full OR space could rapidly speed up our understanding of receptor/odorant recognition. Importantly, the system provides the potential for subsequent retrieval of individual OSNs for RT-PCR and analysis of gene expression. This study paves the way for further exploration of rare cell types, such as OSNs that respond to pheromonal compounds [48–52].

MATERIALS AND METHODS

Photolithographic Microfabrication of Master Molds

The master molds for our microwells and microfluidic channels were manufactured at the Washington Technology Center Microfabrication laboratory at the University of Washington. All masters were made on test grade 3 in. silicon wafers (Silicon Sense Inc., Nashua, NH) and followed the same protocols as previously reported [39]. Briefly, SU-8 2015 and SU-8 2030 (MicroChem Corp., Newton, MA) were used to generate the microwells and the microfluidic channels, respectively. Two photomasks, one for the microwells (printed at 20,000 dpi, CAD/Art Services, Poway, CA) and one for the microfluidic channels (printed at 6,000 dpi, University of Washington Printing Services, Seattle, WA), were used to expose UV light through our patterns onto SU-8 photoresist that had been spun onto two separate silicon wafers. Wafers were pre-baked to solidify the photoresist, exposed to the patterns, post-baked to selectively crosslink the exposed areas of the photoresist layer and the uncrosslinked resist removed by immersion in SU-8 developer (MicroChem Corp.). After drying, the master molds were exposed to a vapor of (tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-trichlorosilane (Sigma) in moderate vacuum to facilitate the release of the PDMS mold after curing.

Replica-Molding of Masters in Poly(dimethylsiloxane) (PDMS)

Standard soft lithography techniques [33] were used to generate the microwells and microfluidic channels for our device, as previously described [39]. Microwells were generated from a master, cut out of the mold and the microwells replica mold was placed with the wells in direct contact with the floor of a 60 cm petri plate. The plate was then filled with PDMS (covering the microwells), degassed and cured at 65 C overnight. The purpose of this step was to generate a smooth, unbroken surface to bond the microwell layer to our microfluidic layer. The embedded wells were cut out of the PDMS in a 38 × 75 mm block and bonded to a microscope slide (Erie Scientific, Portsmouth, NH) with oxygen plasma

(Plasma Preen II, North Brunswick, NJ). The microfluidic layer was replica molded with PDMS in the same manner as our microwells. Prior to pouring the PDMS onto the master mold of the microchannels, 1–2 cm long pieces of silicone tubing (1.14 mm ID, Cole-Palmer, Vernon Hills, VT) were glued onto the surface of the master mold to serve as entry channels for the device. Upon PDMS curing, the silicone tubing bonds to PDMS, becoming an integral part of the microfluidic device. The microfluidic layer was then oxygen plasma bonded to the microwells (Fig. 1B). The microarray occupies an area of $\sim 4 \times 6$ mm and contains $\sim 32,000$ microwells. Each well has a depth of 10 μm and a diameter of 20 μm . The array has a hexagonal arrangement, with a center-to-center distance of 30 μm .

Olfactory Sensory Neuron Culture and Fluo4-AM Loading

A single Female Swiss-Webster mouse (Harlan Spargue Dawley, Ohio; 6 weeks–3 months of age) was euthanized by CO_2 asphyxiation and both nasal hemispheres were dissected out. The olfactory epithelium lining the turbinates was removed in ice-cold dissociation media (DM) with Kynurinic acid and Magnesium under a dissection microscope and cut into $\sim 1 \text{ mm} \times 1 \text{ mm}$ sections. Once cut, DM was removed and replaced with DM containing 40U of papain latex (Worthington Labs, Lakewood, NJ) and 4.5 mg of L-cysteine. The pH of the solution was adjusted by adding 0.1N NaOH or HCl until the color of the indicator was red (~ 7.4). The tissue was incubated at 37 $^\circ\text{C}$ for 15 minutes on a rotating shaker (100 rpm) and passed through two Falcon (Becton Dickinson, USA) cell strainers (40 μm diam. mesh). An equal volume of Dulbecco's Modified Eagle's Media (DMEM, Sigma) with 20% bovine serum albumin (BSA, Sigma) and trypsin inhibitor (Sigma) were added to the cells to stop the papain enzyme. The cell suspension was incubated for 20 minutes with Fluo4-AM (1 μM final concentration; Molecular Probes) and Pluronic F-127 (0.02% final concentration; Molecular Probes) in the dark at room temperature. Cells were spun down at 1,000 rpm for 5 minutes in a centrifuge (Forma Scientific Inc., Model 5678) and the supernatant removed. Saline was used to resuspend the pelleted cells in 100 μL s and delivered into the microfluidic device. DM consisted of 81.8 mM Na_2SO_4 , 30 mM K_2SO_4 , 5.8 mM MgCl_2 , 252 mM CaCl_2 , 100 mM HEPES and 20 mM Glucose. Phenol red was at a final concentration of 0.00001%.

Odorants and Solutions

All chemicals were purchased from Sigma-Aldrich, unless otherwise indicated. Olfactory epithelial cells were suspended and maintained throughout the experiment in an external saline solution (NaCl, 140 mM; KCl, 5 mM; CaCl_2 , 2 mM; MgCl_2 , 2 mM; HEPES, 10 mM; Glucose, 10 mM; Sucrose, 6 mM). Odorants (vanillin, geraniol, ethyl butyrate and benzyl acetate) were dissolved in DMSO at a stock concentration of 1 M. Odorants were diluted in the external saline solution at 200 μM . Cells were depolarized with an external KCl solution (NaCl, 55 mM; KCl, 85 mM; CaCl_2 , 2 mM; MgCl_2 , 2 mM; HEPES, 10 mM; Glucose, 10 mM; Sucrose, 6 mM).

Operation of the Microfluidic Device

Microwells tend to trap air bubbles due to the high hydrophobicity of PDMS. To avoid trapped air bubbles, we used the described method of Rettig and Folch [39] to fill our device with saline. Briefly, atmospheric air is exchange for CO_2 inside the device. While the device is under CO_2 gas, the device is filled with saline. The CO_2 gas has a lower solvation point than oxygen or nitrogen and the bubbles quickly dissolve into the saline solution, wetting the microwell surface. Olfactory epithelial cells are introduced into the device by capillary flow and deposited into the wells via capillary flow. A female luer lock (Perkin-Elmer, Waltham, MA) is used as a reservoir for the cells. The luer lock is placed on the exit port of the device (Fig. 1B, lower port) and filled with the cell suspension. The lateral channels are pinched off with flat-nosed clips (Radio Shack), leaving the central channel opened. A Kim-Wipe is

applied to the entry port of the central channel to draw the fluid through the channels, generate flow and draw the MOE cells into the central channel. Flow is interrupted for 10 minutes to allow the cells to deposit into the wells. This process is repeated two more times to get all the cells through the device and loaded into the wells. Cells are then allowed to rest for 20 minutes, without flow. Eight 3-mL syringes with female luer locks were connected to the device via silicone tubing (1.14 mm ID, Cole-Palmer, Vernon Hills, VT). The syringes were raised 1 cm above the device in a holding rack, filled up to the 3 mL mark with odorants, saline, KCl (85 mM) or saline with DMSO. Flows were gravity-driven and regulated by pinching the tubing closed or open with flat-nosed clips. Odorants were released into the device in the sequence shown in Fig. 2. The number 0 is the saline wash. Number 1 is the DMSO control. Number 2 is the Vanillin solution. Number 3 is the Geraniol solution. Number 4 is the ethyl butyrate solution. Number 5 is the benzyl acetate solution. Number 6 is the KCl solution. All odorants are at 200 μ M and KCl at 85 mM. The solutions were released in the following order: (0, 1, 0, 1) - 0 - (0, 2, 0, 2) - 0 - (0, 3, 0, 3) - 0 - (0, 4, 0, 4) - 0 - (0, 5, 0, 5) - 0 - (0, 6, 0, 6) - 0.

Microscopy and Image Acquisition

Images were acquired with a Hamamatsu Orca HR (Hamamatsu Photonics, Hamamatsu City, Japan), 10 mega-pixel, cooled-CCD camera at 4x magnification, mounted onto a Nikon Eclipse inverted microscope. Images were captured in time-lapse sequence and processed with MetaMorph software (Molecular Imaging).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Olfactory sensory neuron (OSN) screening platform. (A) A cell bolus is gently flowed into the assembled device (top). Fluid flow is stopped to allow cells to deposit into the wells of our device (middle). Flow is resumed with saline to remove non-adhered cells (bottom). (B) An assembled device is shown with microwells on the bottom layer. The device is filled with a red dye for better contrast. The inlets are numbered in the same order as in the perfusion of the experiment described in the main text. The large black dashed square represents the viewable area (22.9 mm^2) available to our camera (Hamamatsu ORCA-HR) at 4x magnification on our microscope. The region represented by the small solid black square is shown in (C) for a filled device with OSNs deposited in wells. The inset shows a zoomed view of an OSN in a microwell. Red arrows, direction of fluid flow. Black arrow, section of microwells blown up for (c).

Figure 2.

Experimental procedure and cell detection inside the microfluidic device. (A) A representative time line and exposure procedure for our experiments. Blue boxes represent the time blocks where odorant application occurs. Between exposures, saline is continuously flowed over the cells. (B) The wash-in/wash-out times of the microfluidic device were tested with fluorescein (5 mg/mL) dissolved in saline. The KCl exposure profile was used to test the fill and emptying time. (C) A single trace of a user-selected cell responding to KCl (85 mM). OE cells were loaded with Fluo4-AM, a calcium-sensing dye. Arrow heads with number represent the numbered images below the graph. Far left and in a white circle, phase-contrast image of the OSN in a well. (D) Response profiles of user-selected cells during KCl exposure. The traces represent OSNs picked by an expert user and generated using Metamorph analysis software. An average trace of the 33 cell responses (thick, red line in (D)) is generated. (E) A MATLAB template-matching program finds the correlation between each pixel's fluorescence time course and the average trace from (D), generating a grayscale correlation map (left); next, another MATLAB sub-routine detects which of the high-intensity pixels in the correlation map are inter-connected (since these must be the cells) and generates a map with all the centroids of the interconnected shapes (center); a virtual array (right) of responsive cells is generated for human confirmation or removal. The centroid coordinates from confirmed cells are used to generate regions that Metamorph uses to measure the fluorescence changes in odorant-exposed OSNs. *Color scale & Grayscale: black - no fluorescence change; white – highest fluorescence change. Green dashed line, start of stimulus. Red dashed line, end of stimulus.*

Figure 3.

Response profile of individual OSNs to odorants. Individual OSN traces of the highest and lowest responders for each group of odorant combinations (table, right) are displayed. The number of human-confirmed odorant-responsive cells is displayed on the far right column. Each trace pair is calibrated to the same Y-axis (% change in baseline fluorescence, $\Delta F/F$) represented on the top left. The cut-off for inclusion is set at 2% of baseline. All traces are normalized to the first imaged frame in each odorant exposure. An extra exposure to KCl immediately after the single exposure to ethyl butyrate is not shown for clarity, but all cells responded.

Figure 4.

Overlaps in OSN activation in response to odorant exposure. Individual cells responded to the molecules depicted above. All 15 possible combinations of responses are present. The different bar diagrams depict the total number of cells that responded to each odorant, organized into whether they responded to 1 (“X only”), 2 (“X+1”), 3 (“X+2”), or 4 (“X+3”) odorants. (**V**-Vanillin; **G**-Geraniol; **EB**-Ethyl butyrate; **BA**-Benzyl acetate).