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Olfaction on a Chip

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Highlights

- Olfactory sensory neurons are cultured on integrated circuits for odorant sensing.
- The circuit provides on-chip signal amplification.
- The sensor shows consistent and selective response to specific odorants.
- This technology offers the possibility for mobile odorant sensing in real-world settings.

Abstract

On-chip sensing of physical signals is now relatively straightforward, with on-chip tilt sensors and cell phone cameras now ubiquitous, but sensing of odorants has proven to be far more challenging. A general purpose, mobile electronic nose would address pressing needs in security and food safety. We demonstrate here an approach that combines living olfactory sensory neurons (OSNs) with integrated circuitry. The hybrid bio-electronic chip harnesses the as-yet unparalleled capabilities of olfactory sensory neurons – in variety, selectivity, and ability to function in the real world – and takes advantage of modern silicon chip technology for detecting and processing electrical signals. OSNs were cultured directly over an array of electrodes on the surface of a custom integrated circuit so that the signals, a series of "spikes" upon odorant binding, could be locally detected and processed. Consistent and selective responses to specific odorants were recorded from individual neurons.

1 Introduction

Spock's tricorder is still science fiction. While microchip-based physical sensors, such as cameras and pressure sensors, are widespread and particular chemical species can be identified with analytical instruments, we routinely rely on biological noses (dogs, humans) for a general-purpose sense of smell. A mobile "nose on a chip" [1-3] could have potentially transformative applications, from homeland security (such as identification of individuals) to search and rescue or food origin (Greek versus Italian olive oil).

Cell-based olfactory sensors [4-7] offer the eventual promise of sensitivity, selectivity, and discrimination of competing odors, as well as operation in the real world since cells maintain their own functionality and employ adaptation. Moreover, they could employ a combinatorial arrangement of the rich repertoire (thousands) of naturally occurring odor receptors (ORs) to enable complex odor discrimination [8,9] with low false positive and false negative rates.

Cell-based sensing is gradually becoming practically feasible with advances in technology [10-12], bioengineering [13-16], and ligand-OR pairing [17]. A number of bioelectronic noses based on olfactory sensory neurons (OSNs) have been developed [7,9,13,15,18-20] to exploit the unmatched ability of biological systems for odor sensing, but miniaturization has been a significant hurdle due to the benchtop laboratory equipment and computers required for transduction and odorant identification [16]. Furthermore, it is infeasible to output hundreds of channels of analog waveforms (for electrode arrays each channel requires a dedicated bond-pad and a physical wire going to an external amplifier [21]), and extensive parallel data processing is required as the number of sensors grows [3].

We demonstrate a hybrid system that integrates OSNs with an integrated circuit (IC), utilizing the direct transduction by OSNs of odorant binding events to digital electrical signals (action potentials, or "spikes"). Utilizing the signal processing, computing, and multiplexing capability of very large scale integrated (VLSI) circuits will allow on-chip signal detection and interpretation, providing the potential for a mobile miniaturized system able to evaluate signals from hundreds of sensing sites simultaneously and in real time [8,22].

Complementary metal-oxide-semiconductor (CMOS) technology has already been used to implement high-density "active" microelectrode arrays [23,24]. Furthermore, an IC can be used to recognize spikes in hardware, when and where they occur on the chip surface, and report this information off-chip using just a few wires for subsequent pattern recognition [25,26]. However, the integration of living cells with commercial CMOS technology had significant, unique challenges that had to be overcome for this demonstration, including packaging [27], circuit design, and other issues [28,29]. In this work we apply this technology development to olfaction, integrating a perfusion system and adapting mammalian cell culture and surface treatments to the culture of salamander olfactory sensory neurons on the surface of the chip. The biohybrid system was unambiguously able to distinguish among odors, based on the firing of individual OSNs, consistently over the course of several days.

2   Material and Methods

A low noise, low power CMOS bio-amplifier chip with 20 recording sites, substantially similar to the one used in [27], was designed to record extracellular potentials and was produced in a commercial foundry. The amplifier circuits were based on a design that employed an operational transconductance amplifer with the gain set by capacitive feedback [30]. The first stage, with a gain of 60, amplified the potential difference between a pair of surface electrodes (Figure 1a), rejecting common-mode signals coupled into both electrodes to reduce noise. This was followed by a second stage of 20x amplification, giving a total gain of 1200. A third stage with unity gain buffered the output to drive the load presented by the external data acquisition system (DAQ) (Figure 1b). Chips were obtained as individual dies from the foundry and were packaged to allow contact with fluids and cells [27]. Electrical connections between the chip and the DAQ were provided by a two-tier printed circuit board (PCB).

Primary OSNs from salamanders (*Ambystoma tigrinum*) were used in this work because their odor responses have been extensively characterized [31-33]. (The work was carried out in accordance with Directive 86/609/EEC for animal experiments.) The olfactory epithelium was dissociated (see Supplementary Material for detail), producing a mixture of cell types, of which approximately 10% are OSNs [20]. Figure 2a shows a single OSN, with the sensing cilia at the top. The cilia normally protrude into a layer of mucous in the nasal cavity, where they are exposed to air-borne odorants. Olfactory receptor (OR) proteins that bind particular molecular features of odorants are found on the cilia, one OR type per cell. In response to a sufficient number of odorant binding events the cell fires an action potential or "spike" that travels along the axon to the olfactory bulb (OB) [8,22,34,35]. Activation of a subset of the hundreds of different receptors results in a combinatorial code at the level of OSNs, which is transmitted to the OB for further processing.

Upon plating onto the chip (Figure 2b), the OSNs and other cell types were distributed over the surface, with some cells in close proximity to the sensing electrodes. Removal of the olfactory epithelium requires severing the OSNs at the axons, and the combination of mechanical and chemical treatments used for dissociation induces further cell trauma. Therefore, the tissue was processed as little as possible, and clumps of tissue remained (Figure 2b).

The packaged chip is shown schematically in Figure 2c. The chip, a 3x3 mm square, was embedded in an epoxy handle wafer. This left its surface flush with the package and allowed subsequent photolithography to define the Au/Ti thin-film fan-out connections to the bond pads [27]. The Au also covered the non-biocompatible, electrochemically active Al recording electrodes in the center of the chip. The electrical leads, for transmitting power and data, were insulated within the area of the perfusion chamber by a thin film of parylene. The parylene was patterned to remove it from the center of the chip and the outer perimeter of the handle wafer, exposing the sensing electrodes and the contact pads. Electrical connections to the package were made via spring-loaded pins. (See Supplementary Material.) A Teflon perfusion chamber was fixed with biocompatible silicone adhesive to the handle wafer.

Three odorant mixtures were prepared, each containing 3 odor components at individual concentrations of 200 μM with 1% DMSO in the culture media (see Supplementary Material). The odor concentration was chosen to maximize the stimulation of the OSNs and is within the range of those used by previous studies to stimulate acutely dissociated cells from salamander [36]. The stimulation panel included odors of diverse chemical structure, including esters and alcohols. Also, each odor mixture contained at least one odor shown to produce responses in a large percentage of neurons in salamander at the concentration used in our experiments (e.g. amyl acetate and acetophenone) [36].

3   Results and Discussion

Four experiments were conducted on three separate chips at room temperature. Aqueous odorant exposure began one day after plating. The OSNs were exposed to a series of 3 odorant mixtures, one at a time for 1 minute (Figure 3a). Between mixtures the chamber was flushed, at 5 times the odorant flow rate, for 20 seconds. A solution of elevated KCl concentration administered at the end of the series served as a positive control. Data were collected simultaneously from all 20 channels on the chip at 20 kHz. In the event that one or more channels showed a response to a mixture, then 1-2 hours later the three odor components were separately flowed over the chip, again separated by flushing and followed by the KCl solution.

Although the OSN density in the culture was low, signals were obtained from1-2 sites in each experiment, for a total of 2 sites responding only to KCl and 5 responding to both KCl and an odorant: two OSNs fired in response to acetophenone, two to l-carvone, and one to eucalyptol. Results from a chip with two responsive neurons on two electrode pairs (channels) are shown in Figure 3a. These data were high-pass filtered (f_c = 400 Hz) to remove the DC offset and low frequency fluctuations, which facilitated the identification of spikes. Exposure to a stimulant, whether odorant or high KCl, induced a temporally corresponding series of spiking events above the level of background noise. The onset of the responses and the pattern of firing throughout the stimulus duration was similar to that previously shown in dissociated OSNs [32]. Furthermore, as previously noted for *in vitro* culture [18]*,* we did not observe spontaneous firing from these cells in the absence of stimulant.

The neuron on channel 14 responded only to mix 3, the neuron on channel 15 only to mix 2, and both responded to the high KCl solution (control). Within mix 2, acetophenone (a floral smell) produced a robust response on channel 15 (Figure 3c). Channel 14 responded to a single component of mix 3, L-carvone (spearmint) (not shown). We did not observe responses to any other individual components on either channels 14 or 15. In these experiments, each neuron responded to at most one of the nine odorants contained in the three mixtures; however, all of them spiked in KCl.

In response to odorants, the maximum average firing frequency was 10-19 spikes/second, depending on the sensitivities of the neurons to the odorant and consistent with previously reported firing frequencies for salamander OSNs in response to odorants [32].

In subsequent days the OSNs were again probed with the individual odorants from the mixtures to which they had responded on day 1. The results were consistent, with the same neurons responding to the same odorant, and no others, over the course of 3-4 days.

Four individual spikes in response to odorant, from the several hundred recorded on channels 14 and 15, are shown in Figure 3b. The spikes were triphasic, as expected [21,32], having a small dip in voltage both before and after the main peak. These spikes were taken from the raw data, so the shape and amplitude can be seen without distortions introduced by filtering. The peaks were aligned at V = 0 to allow comparison despite a shifting baseline. The shape and size of the peaks was reproduced in every spike. Peak height depends on distance from the recording site while peak direction depends on neuron orientation relative to the electrodes [21]. The width of

the spikes on channel 15, at 1.5 msec, were typical, while those on channel 14 were unusually thin at 1 msec.

4   Conclusions

There are several next steps needed to move forward from this proof of concept. One is to incorporate on-chip spike recognition in the lab-on-CMOS technology [37-39]. This will greatly reduce the amount of data currently transmitted and eliminate the need for post-processing the data on all the channels to identify action potentials. Instead, the chip will simply report addresses and times (events). This will enable increasing the number of recording sites to the hundreds that will be necessary for general-purpose, portable odorant detection in a system that can be used outside of a lab. Another step is to replace the perfusion well with microfluidic channels (already demonstrated [27]) to allow rapid and precise temporal control over the introduction and removal of odorants [15,40,41], and then eventually adapting the system to handle airborne odorants rather than aqueous solutions. A third critical step addresses the biology: primary cells have a limited lifetime and random placement on the chip. Techniques have been reported for developing primary OSN cultures [42], although this is is not straightforward. Primary cultures of specific OSN types would allow a number of each type to be plated onto known electrodes, recreating a sensing array and architecture similar to the one in the biological nose and providing the redundancy needed to reduce false positives and false negatives. Longer term, cell lines that can be kept in stasis before use or expressing other receptor proteins [13] could potentially be genetically engineered. For example, rat olfactory receptors have been expressed in yeast to obtain an optical response [14], and electrical signals have been obtained by transfecting *Xenopus* oocytes [15,16]. Since the list of known receptor-ligand pairs is growing rapidly [16,20,43], it may become feasible to create the large cross-responsive arrays needed for olfaction.

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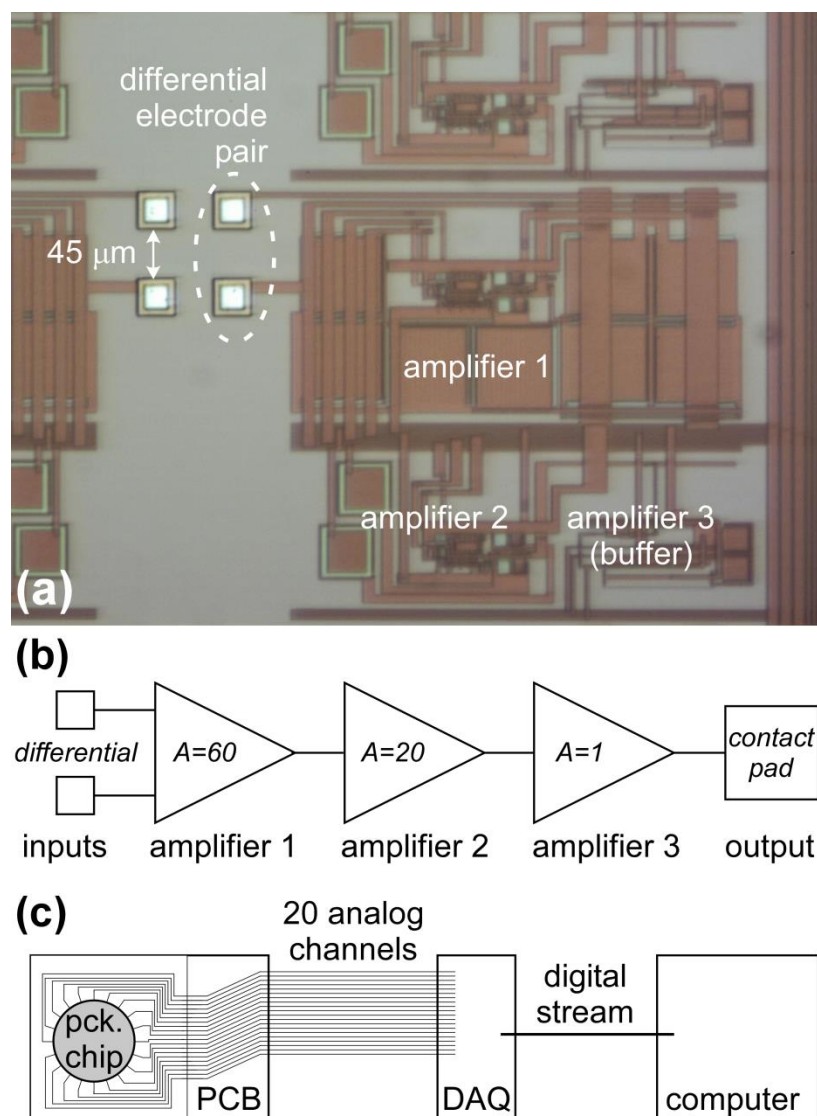
Figure 1. **a)** **Close-up of the chip surface showing two of the recording sites and the circuitry to which one pair of electrodes was connected.** **b) The amplification stages, leading to a total gain of 1200. c) The chip was packaged and interfaced to a printed circuit board (PCB). The PCB was connected to a data acquisition system that converted the analog voltages to digital signals, which were transmitted to a laptop for recording and later analysis.**
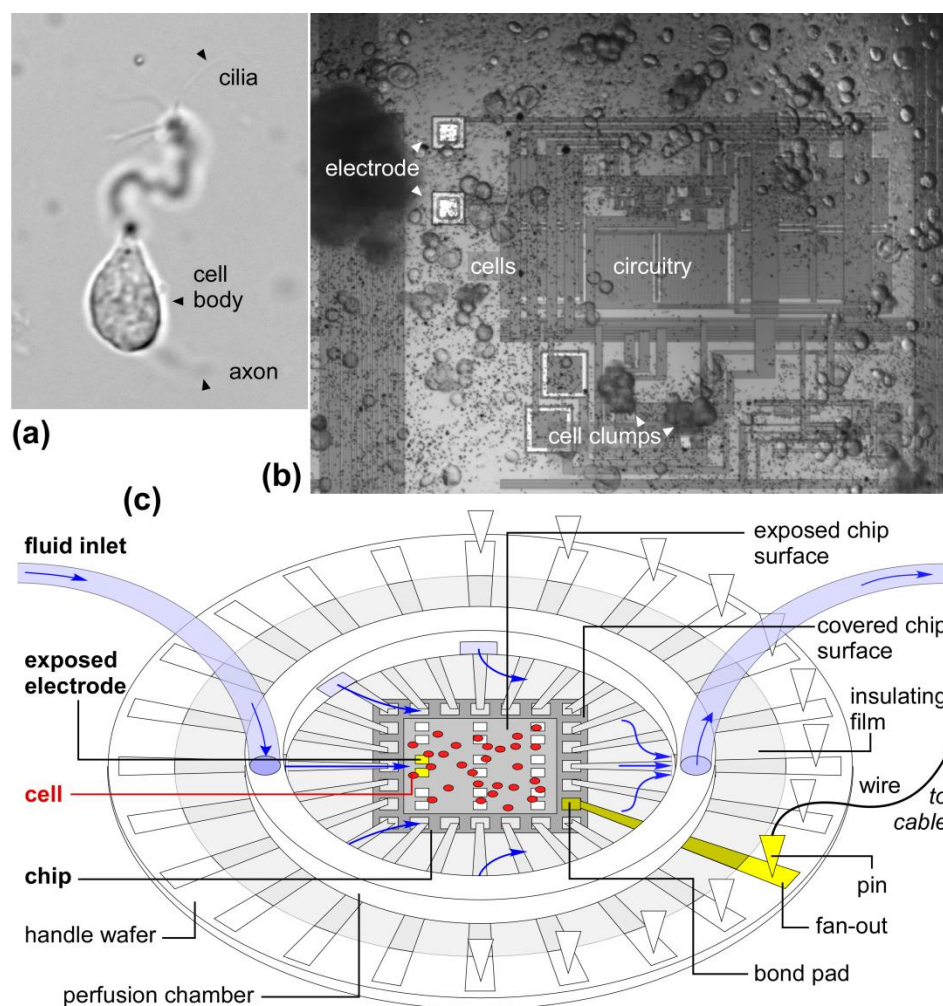
Figure 2. **a)** A dissociated OSN from a salamander showing the cell body and several cilia (topmost arrowhead points to one of them). **b)** Several OSNs and supporting cells dissociated from salamander olfactory epithelium plated onto the surface of one of the chips, shown one day after plating. Circuitry and electrodes (white arrowheads point to an electrode pair) are visible, as well as a variety of cell types and clumps. **c)** Schematic of the packaged system showing the electrode array in relation to perfusion flow (blue arrows).
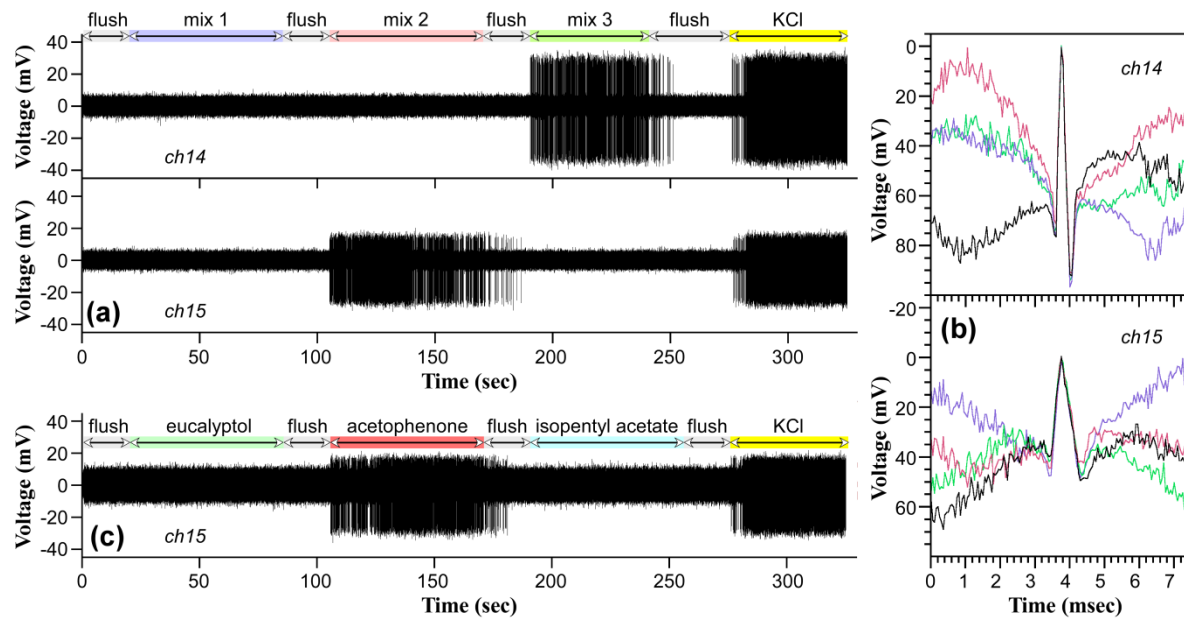
Figure 3. **a) Responses (high-pass filtered) of two neurons (channels 14 and 15) to three odorant mixtures and KCl.** **(b) Four spikes (unfiltered but aligned) from the odorant response spike trains from the two cells shown in (a).** **The y-axes have been reversed.** **c) Response of channel 15 to the individual components of odor mix 2. All magnitudes are post-amplification on chip, a factor of 1200.**