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A novel bioelectronic nose based on brain–machine interface using implanted electrode recording *in vivo* in olfactory bulb

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ABSTRACT

The mammalian olfactory system has merits of higher sensitivity, selectivity and faster response than current electronic nose system based on chemical sensor array. It is advanced and feasible to detect and discriminate odors by mammalian olfactory system. The purpose of this study is to develop a novel bioelectronic nose based on the brain–machine interface (BMI) technology for odor detection by *in vivo* electrophysiological measurements of olfactory bulb. In this work, extracellular potentials of mitral/tufted (M/T) cells in olfactory bulb (OB) were recorded by implanted 16-channel microwire electrode arrays. The odor-evoked response signals were analyzed. We found that neural activities of different neurons showed visible different firing patterns both in temporal features and rate features when stimulated by different small molecular odorants. The detection low limit is below 1 ppm for some specific odors. Odors were classified by an algorithm based on population vector similarity and support vector machine (SVM). The results suggested that the novel bioelectonic nose was sensitive to odorant stimuli. The best classifying accuracy was up to 95%. With the development of the BMI and olfactory decoding methods, we believe that this system will represent emerging and promising platforms for wide applications in medical diagnosis and security fields.

1. Introduction

Since 1982 (Persaud and Dodd, 1982), researchers have made significant efforts to develop technologies, commonly referred to as electronic noses that could detect and recognize odors and flavors. Some of these artificial olfactory systems have fulfilled industrial needs (Berna, 2010) and shown possible and future applications in the fields of health and security (Oh et al., 2011). However, low resolution of chemical gas sensors and complexity of the enrichment and desorption units militate against the widespread use of these devices.

The natural olfaction is still the primary 'instrument' used to detect and discriminate odors, which exhibits both high sensitivity and selectivity. Canine scent detection has been utilized by men for thousands of years. In recent years, rats with sharper sense of smell were trained to sniff out land mine and tuberculosis bacterium in sputum samples (Poling et al., 2011). Generally, this kind of utilizations need long time behavior training and have high failure rate (Gordon et al., 2008). The development of bio-mimetic techniques to detect odorants has been promoted by research in the biological mechanisms of the olfactory system, especially the discovery of gene family encoding vertebrate olfactory receptors (ORs) which can be selectively activated by particular odorants (Buck and Axel, 1991). Since the concept of the bioelectronic nose was first put forward by Gopel (Gopel et al., 1998; Ziegler et al., 1998), our laboratory is focusing on combining olfactory functional components with micro-chips to mimic a biological nose that could supplement the shortcoming of electronic noses and coarse natural olfaction utilization through behavior training. We have developed some kinds of bioelectronic nose based on *in vitro* measurements by microelectrode arrays (MEAs), light-addressable potentiometric sensors (LAPS) and surface acoustic wave (SAW) devices (Fig. S1). Many kinds of biological components originating from the olfactory system have been used as recognition elements in these systems, including olfactory tissues (Liu et al., 2010a, 2010b, 2011, 2012), olfactory cells (Liu et al., 2006; Wu et al., 2009), and olfactory-related proteins (Wu et al., 2011). These systems can be used to detect odorants or research olfactory mechanism. However, as any *in vitro* measurement technology, the significant shortcomings are the rigorous measuring environments and easily inactivated, which restrict specific field-based applications (Du et al., 2012).

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In this paper, we present a novel bioelectronic nose based on BMI without these shortcomings using whole animal as sensing element. We have known all olfactory receptor neurons expressing the same ORs converge onto the same glomeruli in the OB, where terminal axons of ORNs make excitatory synapses with apical dendrites of M/T cells, the output elements of the OB (Rinberg and Gelperin, 2006). Odors are ultimately represented through the action potential activity of M/T cells, whose selectivity and tuning to odorant molecules are therefore fundamental determinants of OB olfactory coding. Taking advantage of this mechanism, we measured neural responses of M/T cells to different odorants *in vivo* by implanted 16-channel microwire electrode arrays and then assessed the smell by neural decoding methods based on population vector similarity. This bioelectronic nose has merits of low cost, less electrodes and less complicated algorithm comparing to other study (You et al., 2011). The results indicate that different odor responses can be recognized preliminarily, which suggests future actual applications of this BMI system.

2. Materials and methods

2.1. Electrophysiology recording and spike sorting

Neural activities from OB were obtained by home-made 16-channel microwire electrode arrays (shown in Fig. S2a) constructed with 35 μm nichrome wire (AM system, WA, USA; #762000), with an impedance of 300–500 kΩ at 1 kHz. Commercial preamplifiers (non-inverting amplifier circuit, 20 ×) were connected directly to the microwire electrode array to reduce electrical artifacts. The data from individual electrodes were amplified by 1800 × gain, filtered in two separate frequency bands (1–300 Hz and 200–5000 Hz) and digitized at 1 and 40 KHz, to obtain local field potential (LFP) and single unit activities, respectively. Spikes were detected by a threshold set at a level 3 times higher than the standard deviation on the high-pass filtered signal, decomposed in 12 features, and automatically clustered by the offline sorter software (Plexon Inc., Dallas, TX, USA).

2.2. Animal preparation and surgery

All of the procedures in this study conformed to the regulations for the administration of affairs concerning experimental animals (1988) and were approved by the Zhejiang University Animal Care and Use Committee. Male Sprague Dawley rats weighting 180–280 g were anesthetized with an intraperitoneal injection of chloral hydrate (4 mL/kg) and maintained with supplemental does delivered once every 2 h or as needed. The animal's body temperature was maintained at 37 °C throughout the experiment. Animals were prepared for recording by exposing the dorsal surface of the OB with a craniotomy and removing the dura mater. The 16-channal microwire electrode array was lowered vertically by a hydraulic pressure-microelectrode propeller (Narishige Group, Japan) in one hemisphere of the dorsal OB (Fig. S2b) until a characteristic large unitary activity was observed (Kay and Laurent, 1999), approximately 300–400 μm which corresponds to the average depth of the mitral cell layer (Bathellier et al., 2008). Recording locations were widely distributed across the dorsal, ventral, medial, and lateral OB to minimize bias from sampling a restricted population. After insertion, blood in the implant site was washed away with saline and the surface of the OB was rinsed with saline to prevent drying. The animal's respiration was simultaneously monitored by recording chest wall movements using a piezoelectric device throughout the course of the experiment.

2.3. Odor delivery and experimental protocol

All reagents were purchased from Sigma-Aldrich (Milwaukee, WI, USA). The main principles of odorant selection are nontoxic, available and with different functional groups. Five small molecular odorants were used for stimulation: anisole, citral, carvone, isobutanol and isoamyl acetate, which are having pleasant smell and easily identified by human olfaction. Odorants were stored in liquid phase (diluted 1:100 in mineral oil) in glass vials at 0 °C, and were brought to room temperature prior to each experiment. Odorants were further diluted with cleaned humidified air to a specific concentration and delivered to freely breathing animals by a commercial olfactometer (PHM-275, Med associated Inc.). The total flow was constant at 0.4 L/min. Anisole, citral, carvone, isobutanol at 10^{-5} M were used to study specificity and odor discrimination of the bioelectronic nose. Carvone and isoamyl acetate were used to detect sensitivity, detection low limit and mixture responses.

The tube port was positioned approximately 1 cm away from the rat nose. Every stimulus was repeated about ten times in turn for one rat. Each trail consisted of 5 s of baseline recording with clean air and 5 s odorant stimulation. Inter-trial interval was 30 s, in which clean air was delivered to exhaust former stimulus. The whole system was shown in Fig. 1.

2.4. Population vector and classification algorithm

Generally, at least ten responsive neurons could be measured by a single microwire electrode array. Population vector algorithm was used to extract information from M/T cell ensemble activity. Each vector element consisted of the normalized spike firing rate of a given neuron. We divided spikes into different successive, non-overlapping fixed time bins (0.1 s, 0.2 s, 0.4 s, 0.5 s, 0.6 s, 0.8 s and 1 s) and breathing cycle bin. Since different neurons discharged inherently in variable ways, we defined the normalized spike firing rate $x_n(k)$ by

$$x_n(k) = \frac{r_n(k) - r_n}{\delta_n}$$

where $r_n(k)$ is the raw spike firing rate of neuron #n in the kth bin, r_n and δ_n are the estimated mean and standard deviation of spike firing rate of 5 s baseline recording before odor stimulation in the same trial.

In this study, support vector machines (SVM) with the radial basis function (RBF) kernel were used for odor classification which is defined as

$$K(x_i, x_j) = \exp(-\gamma ||x_i - x_j||^2)$$

where x_i, x_j are two given training vectors, γ is a parameter that bigger than zero and was optimized with penalty parameter by a grid search using cross-validation method.

One trial per stimulus was randomly chosen to be a testing set, and the remaining trials were used to be the training sets. The length of the vector was determined by the number of neurons times the number of time bins. The percentage of success was defined as the fraction of test trials that were correctly identified. We have detected the effect of various timescales, number of neurons and lengths of decoding time on odor classification performance. The classification success rates under different conditions were shown and discussed in Section 3.

3. Results

In this study, we used 16-channel microwire electrode arrays to record neural responses of OB to various odors from 44