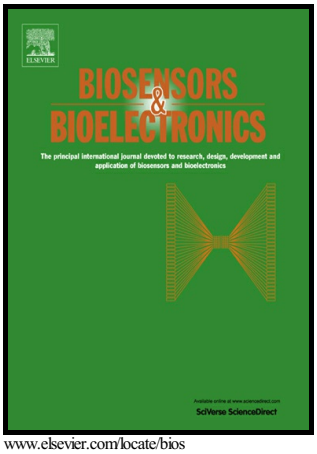

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PII: S0956-5663(17)30591-2
DOI: https://doi.org/10.1016/j.bios.2017.08.055
Reference: BIOS9965

To appear in: *Biosensors and Bioelectronic*

Received date: 12 May 2017
Revised date: 24 August 2017
Accepted date: 25 August 2017

Cite this article as: Keqiang Gao, Songmin Li, Liujing Zhuang, Zhen Qin, Bin Zhang, Liquan Huang and Ping Wang, *In vivo* bioelectronic nose using transgenic mice for specific odor detection, *Biosensors and Bioelectronic,* https://doi.org/10.1016/j.bios.2017.08.055

In vivo bioelectronic nose using transgenic mice for specific odor detection

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Abstract

The olfactory system is a natural biosensor since its peripheral olfactory sensory neurons (OSNs) respond to the external stimuli and transmit the signals to the olfactory bulb (OB) where they are integrated and processed. The axonal connections from the OSNs expressing about 1000 different types of odorant receptors are precisely organized and sorted out onto 1800 glomeruli in the OB, from which the olfactory information is delivered to and perceived by the central nervous system. This process is carried out with particularly high sensitivity, specificity and rapidity, which can be used for explosive detection. Biomimetic olfactory biosensors use various biological components from the olfactory system as sensing elements, possessing great commercial prospects. In this study, we utilized the genetically labeled murine M72 olfactory sensory neurons with the green fluorescent protein (GFP) as sensing components and obtained long-term *in vivo* electrophysiological recordings from the M72 OSNs by implanting the microelectrode arrays (MEAs) into the behaving mouse's OB. The electrophysiological responses showed high reliability, reproducibility and specificity for odor detection, and particularly, the high sensitivity for the detection of odorants that contain benzene rings. Furthermore, our results indicated that it can detect trinitrotoluene (TNT) in liquid at a concentration as low as 10^{-5} M and can distinguish TNT from other chemicals with a similar structure. Thus our study demonstrated that the *in vivo* biomimetic olfactory system could provide novel approaches to enhancing the specificity and increasing working lifespan of olfactory biosensors capable of detecting explosives.

Keywords: Biomimetic; M72-GFP OSNs; MEA probe; Electrophysiological recording; Odor detection; TNT

1. Introduction

The ability to detect odorants is crucial for animals' survival as it provides animals with vital information from their environment: food, approaching predators, nearby mates, and others (Ma 2007). Mammalian olfactory system can detect and discriminate volatile chemicals at relatively low concentrations (approximately one ppt or below) with high sensitivity and specificity. A recent study reveals that humans can discriminate at least one trillion olfactory stimuli, instead of 10,000 odors (Bushdid et al. 2014), which further supports the notion that a biological nose is the most effective odor sensing system. Since olfactory receptor (OR) genes were discovered two decades ago (Buck and Axel 1991), considerable progress has been made in understanding the mechanisms of olfaction. Odors are initially sensed by OSNs that are embedded in the olfactory epithelium (OE). Each OSN expresses one type of G protein-coupled odorant receptor (Buck 1996), and OSNs expressing a particular OR project their axons to two glomeruli in each OB (Mombaerts et al. 1996). Upon odorant binding, ORs activate signal transduction cascades and cause cell membrane depolarization, which eventually leads to the generation of action potentials, carrying the olfactory information into the brain via the OB. Thus, the OSNs are responsible for detecting odorant molecules and transforming the chemical energies into electrical signals (Ma 2007).

Advances in biotechnology and nanotechnology have promoted the development of biosensors that mimic mammalian olfactory system (Gopel et al. 1998; Ziegler et al. 1998). Various biological components from the olfactory system have been used as sensing components in the olfactory biosensors (Du et al. 2013a). Among them, OSNs are ideal odor sensing components due to their distinct response patterns and innate encoding ability (Du et al. 2013b). The OSNs-based biosensors exhibit exquisite specificity, high sensitivity and fast response. However, there still exist some limitations. One of the major challenges is the short lifespan. These biological components are difficult not only to isolate from the olfactory system, but also to cultivate or manipulate; moreover, they are easily biodegradable in an *in vitro* environment. Thus the sensors' shelf-lives are much restricted, which in turn limits the reproducibility of the biosensors (Sankaran et al. 2012).

MEAs are devices that contain multiple plates or shanks to monitor electrophysiological changes, essentially serving as neural interfaces that connect neurons to electronic circuitry. There are two classes of MEAs: non-implantable MEAs that are used *in vitro*, and implantable MEAs that are used *in vivo*. The non-implantable MEAs have been used in the olfactory biosensors in combination with functional cells (Glatz and Bailey-Hill 2011; Marrakchi et al. 2007). However, the implantable MEAs have not yet been used in olfactory biosensors although they may provide a powerful tool for long-term *in vivo* electrophysiological monitoring (Buzsaki 2004; Hochberg et al. 2006).

GFP is a protein that exhibits bright green fluorescence when exposed to the

excitation lights in the range of wavelengths from blue to ultraviolet (Tsien 1998). The GFP gene can be incorporated into the genome of an organism with its expression controlled by a target gene's regulatory sequence. Therefore, in cells where the target gene is expressed, GFP is also produced at the same time along with the target proteins. Thus, GFP is used as a marker for gene expression (Chalfie et al. 1994), and can be used to label OSNs expressing a defined OR gene to help elucidate the cellular mechanisms underlying olfaction (Feinstein et al. 2004; Serizawa et al. 2000).

Here, we use GFP genetically labeled murine M72 OSNs as biosensing components to develop a biomimetic olfactory system. An IRES-tauGFP was inserted downstream of the endogenous coding sequence of olfr160 (M72), resulting in bicistronic expression of the endogenous odorant receptor gene and tauGFP. M72 and tauGFP are expressed simultaneously in the same olfactory sensory neurons, and the fluorescence of GFP can be used to identify the OSNs expressing M72 and the glomeruli they innervate. OSNs with a defined OR can improve the specificity for odor detection (Du et al. 2013b). Unlike the conventional biosensor, we recorded the electrophysiological signals from M72 OSNs *in vivo* using implantable MEA probe. In order to perform long-term monitoring from the intact olfactory system, we recorded the M72 OSNs from the OB upon chronically implanting the MEA probe into the OB of conscious mice. By coupling the genetically engineered OSNs with MEA probe *in vivo*, more natural olfactory responses can be obtained. To examine the efficacy of this novel biomimetic olfactory system, we first explored its reliability, reproducibility and specificity for odor detection.

Further, we investigated its sensitivity and specificity for TNT detection, which has been one of the most widely produced explosives. Explosives have been classified into several types based on their structure and performance. Primary explosives include lead azide and lead styphnate, which are also called initiating explosives because they are used to ignite secondary explosives. Secondary explosives include nitroaromatics and nitramines which are more prevalent at military sites, and TNT belongs to the secondary explosives (Singh 2007).

Sensors based on microcantilever transducers feature superior mass sensitivity, smaller size, lower cost and better compatibility with large multisensor arrays. The microcantilevers produce large bending responses in the presence of TNT vapors or its analogues. The noise, however, limited TNT detection with a threshold estimated to be 520 ppt (Datskos et al. 2003). The biosensor of electrochemiluminescence immunoassay has been developed to detect TNT, in which enzyme-linked antibodies bound to paramagnetic beads are upgraded on an electrode magnetically; its detection limit for TNT was 31ppb under laboratory conditions (Wilson et al. 2003). Compared with these means of detection, *in vivo* bioelectronic nose using transgenic mice for TNT detection has a relative low detection limit and could explore the location of the bomb in a certain circumstance.

2. Material and Methods

2.1. MEA probe for electrophysiological recording

Implantable MEA probe is a utility multichannel sensor that can simultaneously

record electrophysiological signals from many neurons distributed spatially across the brain. It was implemented on Silicon-On-Insulator (SOI, $Si/SiO_2/Si$, 30 μm/1.5 μm/200 μm) wafer by Micro-electro-mechanical System (MEMS) fabricating technology. The fabrication process is illustrated in Fig. 1a, including the following steps: Step 1: SOI wafer was cleaned and coated with a layer of thermally grown silicon dioxide; Step 2: the conducting layer (Cr/Au, 50 nm/250 nm) was deposited and patterned using lift-off technique; Step 3: a layer of silicon nitride (1.1 μm) was deposited by Plasma Enhanced Chemical Vapor Deposition (PECVD) for insulation, and then Reactive Ion Etching (RIE) was used to expose recoding sites and bonding pads; Step 4: deep RIE process was used to define the probe geometry; Step 5: the individual MEA probe was released from the underlying silicon substrate by wet etching. Each MEA probe contained two 6-mm long shanks, and had 16 recording sites (15 μm in diameter), or four tetrode groups. The distances between recoding sites are illustrated in Fig. 1b.

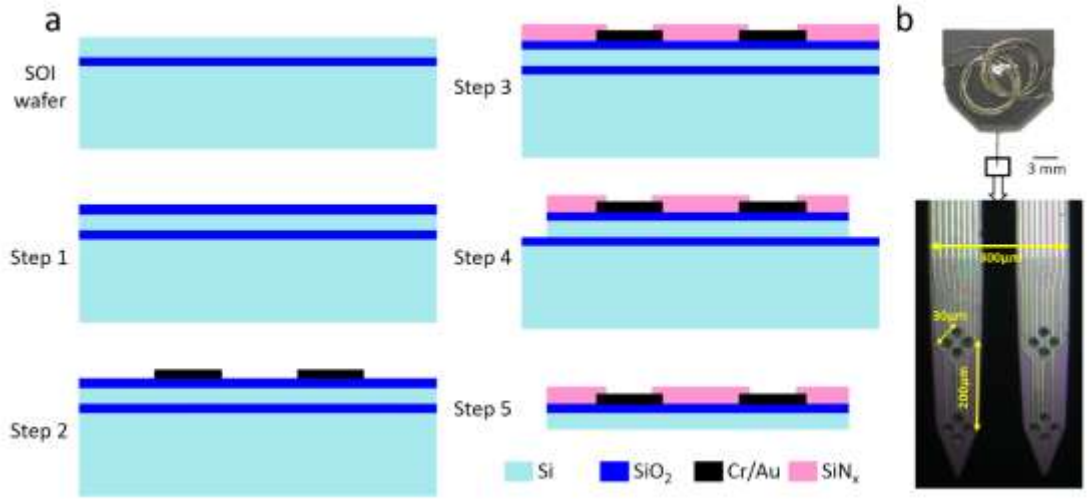

Fig. 1 MEA probes. (a) Schematic of the MEA probe fabrication process. (b) Encapsulated MEA probe (top) and photomicrograph of Pt black modified probe tip

(bottom). Pt black can lower the impedance of microelectrodes and improve the signal-to-noise ratio of electrophysiological recordings.

2.2. OSNs expressing M72 receptor

Detection and discrimination of odors by OSNs critically depend on a large family of G protein-coupled, seven transmembrane ORs. Current research suggests that OSNs express ORs and detect most of odorant molecules via a G protein coupled signaling pathway: upon the binding of odorant molecules specifically to the OR on the OE, the receptor changes its conformation and activates a heterotrimeric G protein, G_{olf}; the G protein in turn activates the downstream effector, adenylate cyclase III (AC3), which produces the second messenger cAMP, leading to the opening of cycilc nucleotide-gated (CNG) ion channels, followed by the depolarization of the cell membrane and generation of action potential (Munger et al. 2009) (Fig. 2a). Each OSN expresses one type of OR, and axons from OSNs expressing the same OR converge onto defined glomeruli in the OB (Fig. 2a). To visualize the expression of OR on OSNs and to record from the OSNs expressing the particular receptor in the OB, we used the transgenic mice expressing tau-GFP under the promoter of a specific OR gene. We chose the M72 OR gene primarily because the axons of M72-expressing OSNs project to the glomeruli in the dorsal OB, an area that is readily accessible for anatomical and physiological investigations (Potter et al. 2001) (Fig. 2b). The M72-IRES-tauGFP mice were constructed in which M72 coding region is followed by an IRES and tau-GFP (Feinstein and Mombaerts 2004) (Fig. 2b). The specific

glomeruli in the OB were identified by the fluorescence of GFP (Fig. 2c). The mice used in this study were in a mixed 129×C57BL/6 background.

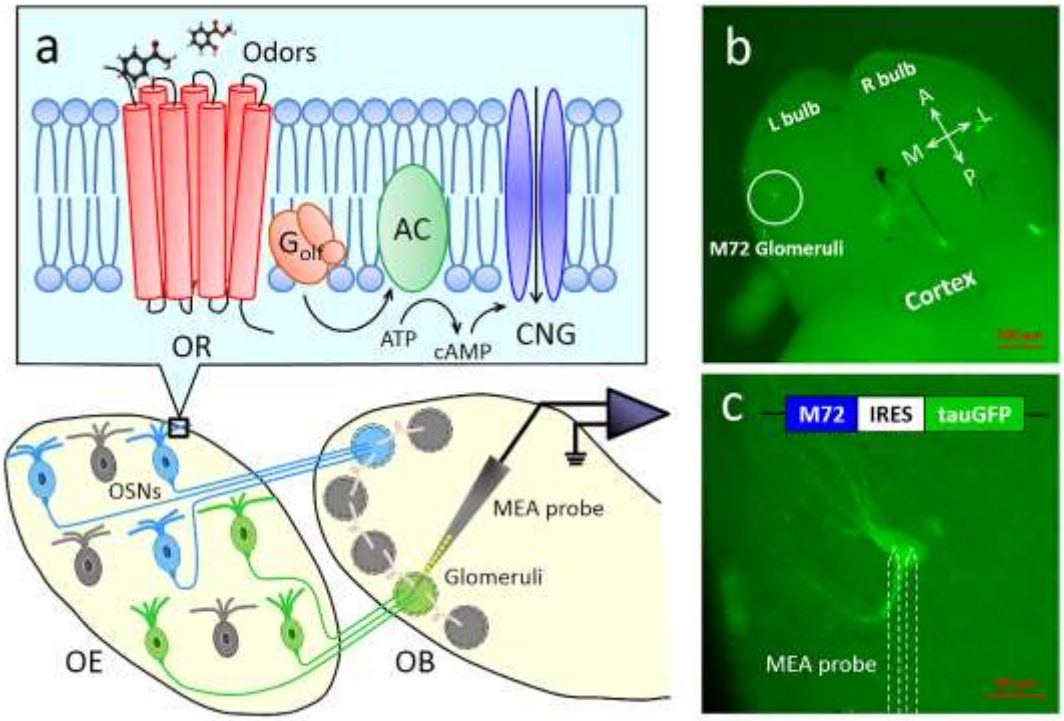

Fig. 2 MEA recordings from behaving mice. (a) Schematic of the experimental method for in vivo OSNs recordings in the murine OB (bottom). The G protein/cAMP pathway underlies signal transduction in the OSNs (top). (b) Axons of M72-GFP OSNs in the OB were recorded by implantable MEA probe under the guidance of fluorescent imaging. The labeled axons coalesce into defined glomeruli in the dorsal OB. A: anterior; P: posterior; L: lateral; M: medial. (c) Section of OB containing mature M72-GFP glomerulus.

2.3. In vivo recordings of M72 OSNs from OB

Male adult M72-IRES-tauGFP mice (6–12 weeks old with a range of bodyweight of 20–30 g) were used for *in vivo* recording. During electrode implantation process,

the mouse was anesthetized with pentobarbital (50mg/kg body weight) and fixed in a stereotaxic apparatus. The skull above the dorsal surface of the OB was thinned through a small craniotomy, and the M72-GFP glomerulus was identified under an upright microscope (Navitar 1-15800) equipped with a CCD camera (Infinity 2). Under the guidance of fluorescent imaging, a MEA probe was targeted toward an M72 glomerulus with an angle of 60° to the pial surface (Fig. 2b). The probe mounted on the micromanipulator (Narishige, Inc.) was lowered gradually to a depth of ~200 μm from the surface (Potter et al. 2001), and was chronically fixed onto the mouse's head by dental cement. All experiments were performed in accordance with a protocol approved by the Zhejiang University Animal Care and Use Committee.

After 4–5 days' recovery, electrophysiological recording was acquired by attaching the connector of MEA probe to a pre-amplifier with a headstage cable connected to OmniPlex Data Acquisition System (Plexon, Inc.). Signals were amplified 1000× gain, sampled at 40 kHz, filtered between 0.5 and 8000 Hz, and stored on a computer. Odors (Aladdin Chemical Co., Ltd.) including TNT, acetophenone, methyl salicylate, citral, diacetyl were used for stimulation. The concentration of the standard TNT solution was 1 mg/ml, whose molar concentration was 4.4 mM. All of the odors were diluted in mineral oil to desired concentrations. During stimulation, odors were delivered to mouse snout for 5 s. Each odor was presented for consecutive trials with an intertrial interval of ≥1 min to reduce habituation (Wilson and Sullivan 1992).

2.4. Data analysis

Neural signals were analyzed off-line in MATLAB (The Mathworks Inc.). First, action potentials (200–4000 Hz) were extracted by band-pass Butterworth filter. For a given epoch of signal, the distribution of spikes was achieved using a dual threshold method. The 3×standard deviation (SD) value of spike signals was set as the level of baseline noise. The spike was taken if the signal exceeded 1.5×SD in the positive direction and exceeded -3×SD in the negative direction. Once the distribution of spikes was achieved, each spike was timestamped. The time values were used to calculate firing rates and peristimulus time histograms (PSTHs). PSTHs were computed by counting the number of spikes with sequential time bins (500 ms), which were used to visualize the firing rate before and after stimulus. Single neurons were classified based on waveform shape. For each neuron, we calculated its mean spontaneous firing rate f_{spon} before stimuli and mean odor-evoked firing rate f_{odor} after stimuli. The odor-evoked response of each neuron was defined by firing rate change ratio, that is $\Delta f/f_{spon}$ ($\Delta f = f_{odor}-f_{spon}$). The magnitude of Δf was calculated by subtracting the mean firing rate of spikes before odor onset from the mean firing rate of spikes that occurred (during 5 s) after odor onset. Student's *t*-test was used as an estimation for significance ($P<0.05$).

3. Results and Discussion

3.1. Identification of the M72 OSNs in the OB

The OB has a multi-layered cellular structure. The layers from surface to the center are: glomerular layer, mitral/tufted (M/T) cell layer and granule cell layer. The glomerular layer receives direct input from the OSNs, and each glomerulus is

populated exclusively by the axons of the OSNs expressing the same OR. In this study, we recorded the axons of the M72-expressing OSNs in the glomerular layer. The search for M72 OSNs was done in an anesthetized mouse by visualizing the fluorescent markers with a microscope and by advancing the MEA probe into the OB in microsized steps with a micromanipulator. The identity of M72 OSNs was established by the following criteria. (1) Strong visible green fluorescence on the surface of the OB. The M72 genes are expressed in the OE, and axons of M72-expressing OSNs project to the defined glomeruli in the OB (Fig. 2c). In whole-mount epifluorescence microscopy, the GFP-labeled glomeruli are located at recognizable, bilaterally symmetric positions in the medial and lateral hemispheres of the OB (Potter et al. 2001). MEA probe was targeted to the medial-dorsal GFP under the guidance of fluorescent imaging (Fig. 3a). (2) The MEA probe was lowered into the glomerular layer in ~200 μm depth from the surface according to the layered structure of the OB. (3) Spikes recorded from the axons of the M72 OSNs were weaker than than that from the M/T cells. Fig. 3b shows spontaneous spikes in the glomerular layer (210 μm depth) recorded by each channel of MEA probe. Spikes were clustered and displayed in 2D coordinates. A group of similar waveforms was considered as being generated from a single neuron, and single neurons could be separated from multiunit activity (Fig. 3c). The waveforms' peak-to-peak amplitude ranged from 40 to 80 μV. However, the spike amplitude of M/T cells (450 μm depth) ranged from 100 to 400 μV (Supplementary Fig. S1). The results show M/T cell layer generates larger spikes (3–10 folds) than those by the glomerular layer. This is

because the M/T cells are larger than other cells in the OB, thus producing larger spikes (Chaput et al. 1992). According to the above criteria, it is confirmed that the axons of the M72 OSNs could be recorded stably by the MEA probe.

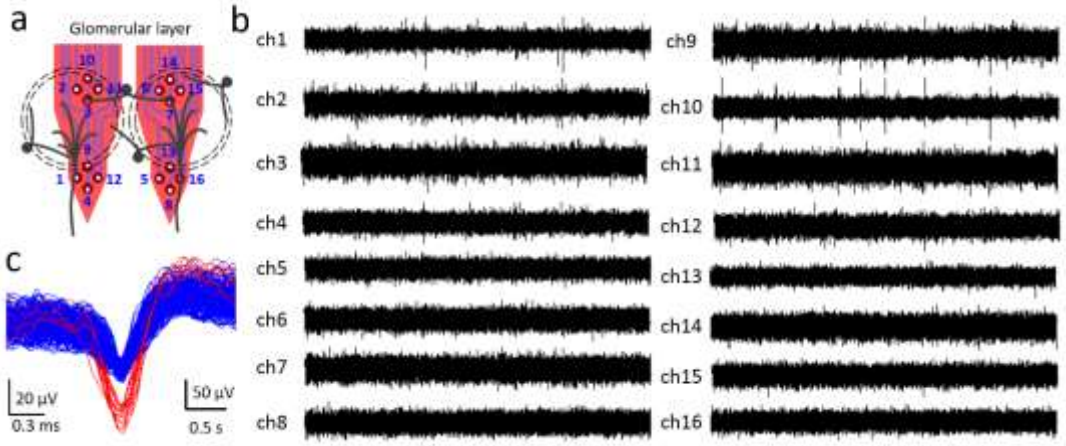

Fig. 3 Spontaneous neural activity recorded from the axons of M72-expressing OSNs in the glomerular layer. (a) Schematic positioning of the MEA probe during recording. (b) A short epoch of raw spike recordings from each channel of the probe. (c) Two single neurons were isolated from the recorded multiunit activity of channel 1 in (b).

3.2. M72 OSNs show high sensitivity to odors containing a benzene ring

A single OR can broadly, yet selectively, bind to multiple odors via relatively weak hydrophobic and van der Waals interactions. Thus a single OSN expressing only one OR-type can respond to multiple odors with different sensitivity. Patch clamp recordings from the knobs of OSNs in the epithelial explants from the M72 mice reveals that M72 OSNs are strongly activated by aromatic compounds methyl salicylate and acetophenone, and they also responds to a series of monoterpene ketone and aromatic esters with less sensitivity (Zhang et al. 2012). Based on these findings,

we first examined the odorant-evoked firing of action potentials from the axons of M72 OSNs in the OB of conscious mice. Using an MEA probe, we recorded both spontaneous and odor-evoked activity of M72 OSNs. In the absence of odor stimulus, each neuron was spontaneously active with a unique firing rate (Fig. 3b). During odor presentation, the firing rate changed. As expected, the recorded neuron was sensitive to acetophenone and methyl salicylate at concentrations of 1 mM, but it responded to diacetyl and citral with lower sensitivity (Fig. 4a). Its mean spontaneous firing rate was 1.8±0.8 Hz, and the firing rate increased to 6.5±1.5 Hz, 4.5±1.5 Hz, 3.4±0.6 Hz, 2.8±1.5 Hz, respectively, in responses to these four odors (Fig. 4b)

Furthermore, we summarized the responses of seven neurons simultaneously recorded by an MEA probe to these four odors (Supplementary Fig. S2). The response of each neuron was reproducible for repeated stimulation with the same odor. Raster plots for single trials and peristimulus time histograms (PSTHs) were created to visualize the firing rate before and after stimulation. For each neuron, we calculated its mean spontaneous firing rate f_{spon} and mean odor-evoked firing rate f_{odor} during odor presentation. Then the change in its firing rate ratio $\Delta f/f_{spon}$ ($\Delta f = f_{odor}-f_{spon}$) could be obtained, and evaluated as response feature of each neuron. Fig. 4c illustrated the average response of seven neurons to four odors. These seven neurons were recorded by a single MEA probe whose action potentials possessed higher signal-to-noise ratios, and were numbered N-1 to N-7. The magnitude of each axis in the figure indicated the change percentage in firing rate ratio ($\Delta f/f_{spon}\times100\%$) in each neuron. The contour of these polar plots differed from one odor to another. However, acetophenone and

methyl salicylate, both containing a benzene ring, evoked more robust responses. So we concluded that the results not only illustrate that M72 OSNs in murine OB could be recorded effectively by an MEA probe, but also indicate that M72 OSNs possess high sensitivity for odorants that contain a benzene ring.

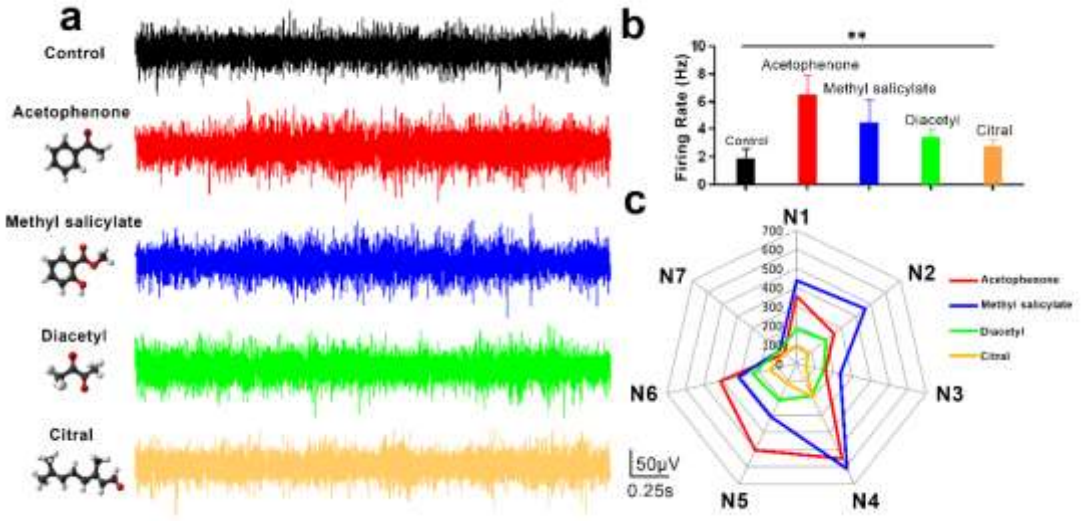

Fig. 4 Recorded action potentials from the M72 glomeruli. (a) Spontaneous and odor-evoked firing of action potentials from a recorded neuron in the M72 glomerulus. The liquid concentration of each odorant is 10 mM. (b) Mean firing rates of spontaneous and odor-evoked activities in (a) were calculated. Significant differences are indicated by $^{**}P<0.01$ that was determined using student's *t*-test. (c) Polar plot of average responses of seven neurons to four odors in terms of firing rate change ratio. The standard deviation (n=10 trials) of firing rate change ratios for the seven neurons ranged between 3.2 and 21.5%.

3.3. TNT detection by M72 OSNs

2, 4, 6-trinitrotoluene, also known as TNT, is one of the most commonly used

explosives for military, industrial and mining applications. TNT is a methylated benzene entity with three NO_2 groups attached to it. According to the above results, M72 OSNs may possess high sensitivity for odors containing a benzene ring. We thus explored the possibility of M72 OSNs as sensitive components for TNT detection. Standard TNT solution was purchased from a chemical company. Its concentration was 4.4 mM. We first analyzed the response of M72 OSNs to the standard TNT solution. As shown in Fig. 5a, during TNT stimulation, not only the firing rate, but also the amplitude of spikes increased dramatically. As expected, TNT at a fixed concentration could elicit robust response in M72 OSNs.

In addition, we investigated the local field potential (1–100 Hz, LFP) activity of M72 glomerulus when detecting TNT. LFPs reflect the activity of a neural network within a volume of tissue. We calculated the average spectrum of LFP before and after stimulation. A significant increase of LFP power in γ band (40–100 Hz) was observed in response to TNT (Fig. 5b). γ oscillation is associated with olfactory coding and plays important roles in odor learning and discrimination. A larger oscillation represents a more activated neuronal assembly near the electrode (Kay et al. 2009). The result further demonstrated that M72 OSNs can respond to TNT.

Animals can sensitively sense the slight changes in odor concentration. In the olfactory neural network, odors evoke concentration-dependent firing patterns that substantially vary in intensity. The dose-dependent responses of olfactory biosensor could be determined by testing different concentrations of odors (Du et al. 2013c). We thus diluted the standard TNT solution into different concentrations ranging from 10^{-6}

M to 10^{-3} M. As shown in Fig. 5c, the firing rates of an M72 OSN decreased with decreasing TNT concentrations. However, when the concentration dropped to 10^{-5} M, its firing rate changed little compared with spontaneity. It is likely that the detection limit was not as low as expected, which may be attributable to the fact that the odor concentration here is referred to as that of odors dissolved in solvent rather than in vapor that was delivered to the mouse snout. Thus, the actual odorant concentration was lower than 10^{-5} M when the odors entered the mouse snout. Because for odor stimulation, we put a filter paper that had been deposited with 0.1 ml odorant solution close to the mouse snout, the odor molecules diffused into air whose concentration was lower than that in the solution. Thus, the actual detection limit of gas concentration was lower than 10^{-5} M. So we determined that its detection limit of liquid concentration was 10^{-5} M. And M72 OSN exhibited dose-dependent responses, which further validated the specificity and concentration range of the responses. In addition, we comparatively analyzed the average responses of multiple neurons to TNT, acetophenone and methyl salicylate. The results showed that different odors elicited diverse response patterns despite their similarities in molecular structures (Fig. 5d).

The impact of the recent terrorist incidents has given rise to increased research interest in faster, more sensitive, more specific and lower cost techniques for explosive detection, especially for TNT (Caygill et al. 2012). Although dogs have long been used to detect explosives and other species are being investigated (Habib 2007), the issues still existing are time and cost of animal training and lack of

quantitative information. To address these issues, biosensors using antibodies, enzymes or whole cells as sensing components have been widely used to detect explosives (Smith et al. 2008). In this study, a novel biomimetic technology for TNT detection has been developed with *in vivo* electrophysiological recording of genetically engineered M72 OSNs. Taking advantage of M72 OSNs' high sensitivity, specificity to odors and MEA probe's long-term recording capability, we expect that this *in vivo* biomimetic system will be a burgeoning biosensor technology supporting future security.

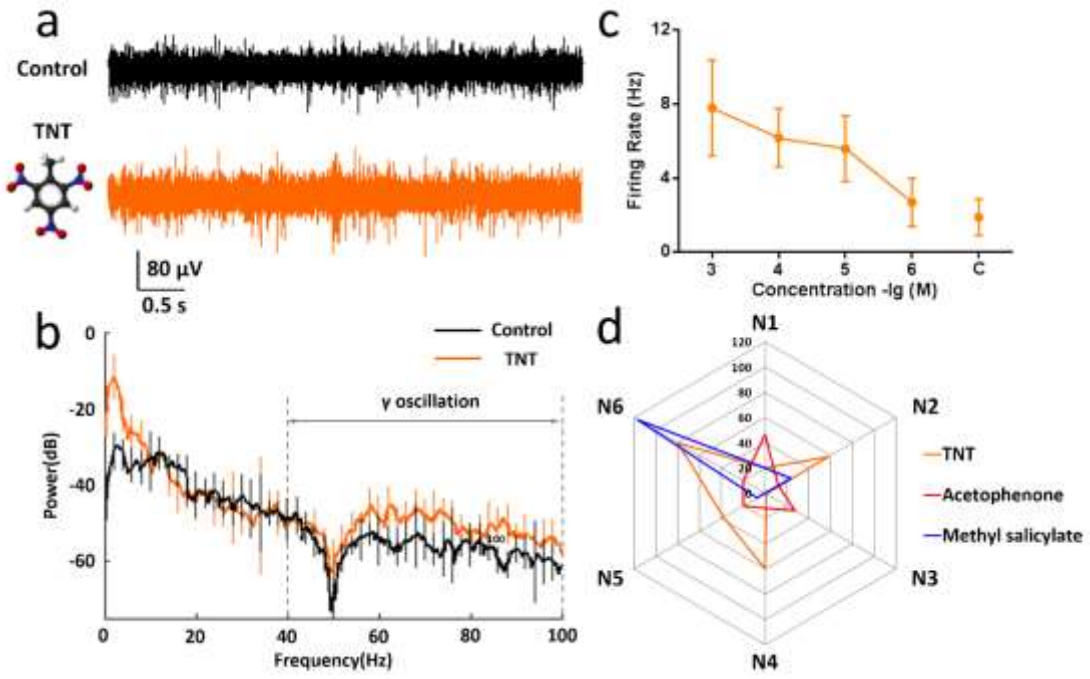

Fig. 5 M72 OSNs' responses to TNT. (a) Spontaneous and TNT-evoked firing of action potentials from a recorded neuron in the M72 glomerulus. The liquid concentration of TNT was 4.4 mM. (b) LFP spectral power before and during TNT stimulation. The solid traces are average power spectrum. Vertical lines represent Jackknife errors at $P<0.01$ (Bokil et al. 2010). (c) The concentration-response curves

to different concentrations of TNT. C is the control group, which represents the spontaneous firing fate of OSNs. (d) Polar plot of average responses of six neurons to three odors in terms of firing rate change ratio. The standard deviation (n=8 trials) of firing rate change ratios for the six neurons ranged between 4.1 and 34.7%.

4. Conclusions

This study presented the design, construction and test of an *in vivo* biomimetic olfactory system for specific odor detection. The genetically engineered M72 OSNs were used as odor sensing components. In order to overcome the limitation of *in vitro* recordings, MEA probe was chronically implanted in the olfactory bulb of a behaving mouse, which allows long-term recordings of extracellular action potentials from multiple M72 OSNs. Our results indicate that this system is a suitable technology for odor detection and identification with characteristically high sensitivity, specificity and stability. Furthermore, it possesses high sensitivity for odors containing a benzene ring, and ability to detect TNT as low as 10^{-5} M with high specificity, showing its great potential in explosive detection. This experiment is a novel attempt to combine transgenic mice and *in vivo* bioelectronic nose for specific odor detection. However，15-μm-diameter electrodes made of gold may possess too high input impedance and generate low signal to noise (S/N) ratios of the recordings. Slightly larger electrodes or electrodes with additional surface coatings could be used to lower the input impedance and to increase S/N in the future work. In addition, further optimization of the experimental procedure, including pre-training the transgenic mice by

familiarizing them with select odorants, as well as more sophisticated analytical algorithms can make their detection even more sensitive and specific, which could lead to a more promising application prospect.

Acknowledgements

This work was supported by Major International Cooperation Project of Natural Science Foundation of China (No. 61320106002), National 973 Project of China (No.2015CB352101), Natural Science Foundation of China (No. 31627801, No. 31661143030, No. 31471008, No. 31628008) and Siyuan Foundation.

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Highlights

1, This paper proposed an invivo odor sensor using OSNs labelled with fluorescent protein, the fluorescent imaging guided the operator to implant the MEA electrodes into neurons to record its electrophysiological signal;

2, The results shown this system possessed high sensitivity for odors which contain benzene ring, and could detect TNT with high specificity, which shown its great potential in explosive detection;

3, The results illustrated that it can detect TNT in liquid concentration as low as 10^{-5} M;

4, It has overcomed the limitation of *in vitro* recording, MEA probe was chronically implanted in behaving mouse olfactory bulb, which allows long-term recording of extracellular action potentials from multiple M72 OSNs.