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Bio-inspired approaches for explosives detection

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ABSTRACT

Due to unique abilities of the animals regarding analysis of complex gas substances, they still remain a gold standard in analysis of explosives. Unusual capabilities of biological chemosensory systems, including both vertebrates and invertebrates, stimulate elaboration of the devices mimicking their activity and operation parameters as precisely as possible. The electronic analogues are a subject of investigation in many research centres, which brings their successful commercialization closer. They are believed to substitute or to complement animals in the analysis of gas substances, including explosive and hazardous ones. The limitations of classic gas sensors can be overcome using the strategies inspired by the solutions known from biological systems. Apart from high selectivity and sensitivity desired for analysis of the explosives, mimicking biological systems allows overcoming other problems connected mainly with effective sampling and odour localization. Presented review is focused on the biomimetic devices, which mimic the sense of smell in a direct way and which are the inspiration to design the devices used for detection of the explosives. Potential of biosensors and bioelectronic noses (B-ENs) to mimic the incredibly accurate and versatile "biological noses" was evaluated. A summary of the strategies inspired by biological olfactory systems should facilitate the approach to the problem of artificial instruments design and to development of the strategy aimed at analysis of the explosives with these systems.

1. Introduction

Biological senses are the examples of adaptive systems, which exhibit unique abilities of perception and reaction to the stimuli present in the environment [1]. Capabilities of the biological chemosensory systems [2] regarding detection, identification and discrimination of complex gas mixtures are the inspiration for elaboration and development of electronic smell analogues [3]. The olfactory systems established during hundreds of years of the evolution can protect organisms against potential hazards *via* identification and discrimination of a wide spectrum of volatile substances. The trends in odour analytics are aimed at utilization of the biological olfactory systems in order to achieve their sensitivity, selectivity and specificity in the electronic counterparts. New generations of sensors and biosensors can overcome some inconveniences of volatile and explosive compounds analysis with commonly used instruments based on gas chromatography (GC) combined with mass spectrometry (MS), ion mobility spectrometry (IMS), Fourier-transformed FTIR, Raman spectroscopy as well as tuneable diode laser absorption spectroscopy (TDLAS) [4]. Moreover, combining (bio)sensors into the arrays opens additional application possibilities [5,6]. The techniques for detection of explosives, including identification and localization of explosives traces, IEDs (Improvised Explosive Devices) and HMEs (Homemade Explosives), should be non-invasive and based on direct identification at the source [7]. Possibility to detect vapours of a given substance is directly dependent on material's volatility [4] (Fig. 1). Sometimes it is necessary to increase sensitivity and reach the ppb/sub-ppb level or lower to meet the criteria of some practical applications (e.g. the drinking water standard for a lifetime exposure to TNT regulated by the United States Environmental Protection Agency is 2 ppb) [8]. Much attention is also paid to durability and sustainable development of the biomimetic materials [9] and

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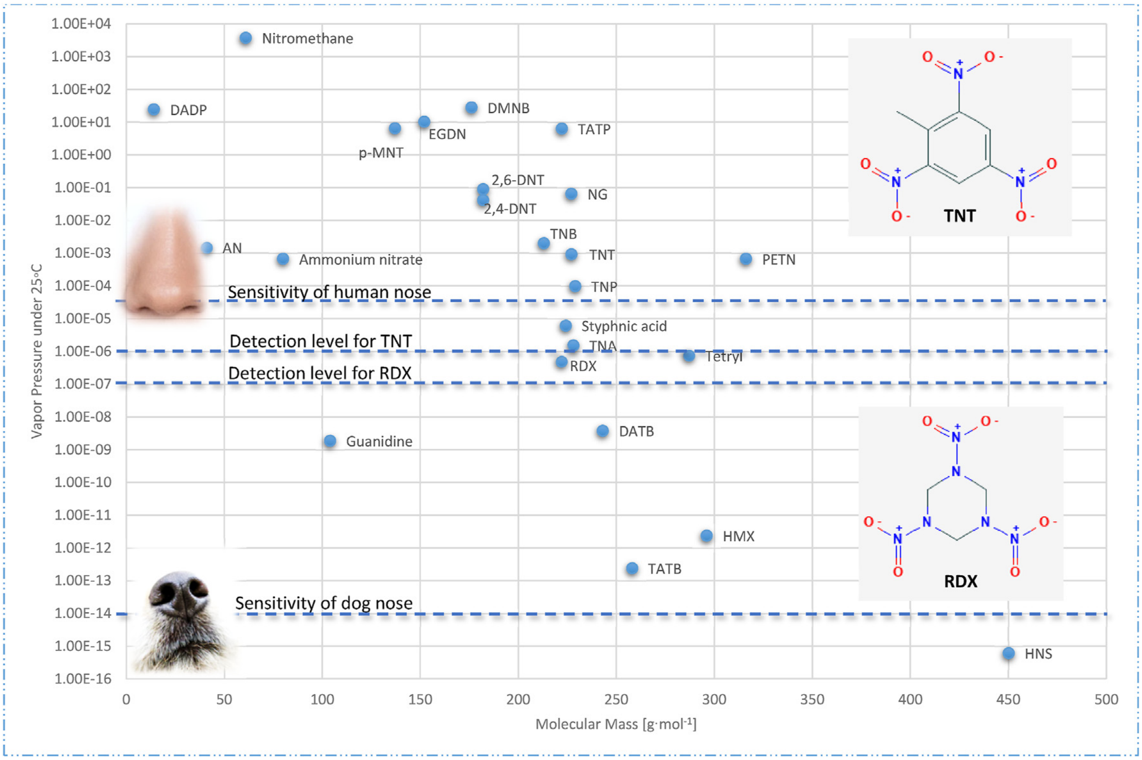
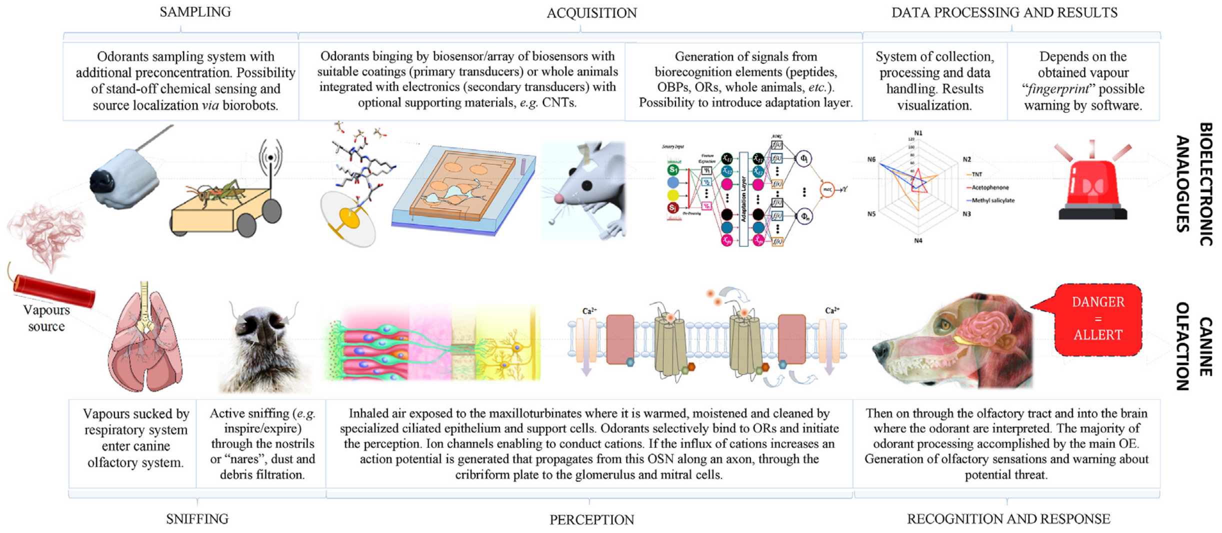


Fig. 1. Scheme of sensitivity levels of humans and dogs noses with detection levels and vapour concentrations of different chemicals with structural formulas of 2,4,6-Trinitrotoluene (TNT) and 1,3,5-Trinitro-1,3,5-triazinane (RDX). Styphnic acid – 2,4,6-trinitrobenzene-1,3-diol, DMNB –2,3-Dimethyl-2,3-dinitrobutane, HNS – 2,20, 4, 40,6,60-Hexanitrophenylethylene, *p*-MNT – 4- Nitrotoluene, 2,4-DNT – 2,4-Dinitrotoluene, 2,4-DNT – 2,4-Dinitrotoluene, 2,6-DNT – 2,6-Dinitrotoluene, TNT –, TNB – 1,3,5-Trinitroben- zene, TNP – 2,4,6-Trinitrophenol, TATB – 2,4,6-Tria- mino-1,3,5-trinitrobenzene, DADP –3,3,6,6-Tetramethyl-1,2,4,5-tetraoxane, TATP – 3,3,6,6,9,9-Hexamethyl-1,2,4,5,7,8-hexaoxacyclononane, EGDN – 1,2-Ethanediol dinitrate, NG – Propane-1,2,3-triyl trinitrate, PETN – 2,2-Bis[(nitrooxy)methyl]propane- 1,3-diyldinitrate, Tetryl – N-Methyl-N-(2,4,6-trini- trophenyl)nitramide, DATB – 2,4,6-Trinitrobenzene-1,3-diamine, HMX – 1,3,5,7-Tetranitro-1,3,5,7-tetrazocane, AN – Acetonitrile, TNA – 2,4,6-Trinitroaniline, Guanidine – 1-Nitroguanidine.

currently their utilization in the explosives detection systems is being intensively developed [10,11].

Commercially available sensors are not selective enough, they are burdened with antagonistic requirements regarding reversibility *vs.* selectivity. In a majority of cases it precludes quantitative measurement of gas traces in different types of mixtures and upon presence of the interferences [12]. Combining the sensors into arrays, initiated by Persaud and Dodd, allowed improvement of the parameters and utility of gas sensors in the complex arrays [5] (Fig. 7.2). The last decades of development of (bio)sensor arrays made it possible to identify the weak points and limitations, which engulf, among others, problems with calibration and recalibration, non-correlated signal drift or sensitivity to the interferents [4]. Moreover, it is critical for their actual implementation for detection of hazardous and explosive substances to increase specificity/selectivity of the (bio)sensor arrays [10]. It should be also emphasized that these devices can provide continuous operation as well as analysis of single samples. Which of the techniques is preferred and implemented depends on a potential application field. Development and progress are also connected with identification speed, size and mass of the device as well as with a recovery time after analysis, which is also related to the lifetime. Additionally, it is important that the device does not require highly trained personnel and is user friendly. Due to biological risks, the systems for explosives detection can be combined with the systems for detection of biological hazard (e.g. viruses) [13]. Sensitivity and selectivity are two parameters describing utility of a device for explosives analysis in a particular application. Hence, most of the strategies of biosensors development concern enhancement of sensitivity and selectivity with respect gaseous analytes. Functionalization of receptor materials of the sensors employed in the arrays allows improvement of selectivity with respect to the compounds having similar properties or similar chemical group. Utilization of suitably selected (bio)sensor array makes it possible to record and interpret the signal, which is similar to a fingerprint. To provide desired selectivity, it is possible to apply a number of the sensors with different functionality in the array, thus offering different response to particular materials. It is the approach similar to the mammalian sense of smell that employs many receptors, which are not selective but general response is interpreted by the brain as a specific odour responsible for adequate olfactory sensation (Fig. 2).

Application of biological or biomimetic receptor components, such as whole animals, bioengineered cells, antibodies, aptamers, peptides, molecularly imprinted polymers (MIPs), olfactory receptors (ORs), odorant binding proteins (OBPs), olfactory sensory neurons (OSNs), as an element of the biosensors allows significant improvement of selectivity/specificity with simultaneous reduction of the problems associated with cross-reactivity and complex sample matrix [10]. A bottleneck in implementation of the biological receptor elements is maintenance of their stability upon connection with the electronic elements. Genetic manipulation and creation of new functional materials *via*, among others, inspiration, mimicking of biosystems and novel combination with transductors, are the further prospects of biosensors development [5,6,14,15]. Most of the biosensors presented in literature is based on identification of the explosives in aqueous phase, soil and on solid materials' surface. Sample preparation, thermal desorption and analyte trapping stages are frequently required [14]. Direct



Odorants sampling system with additional preconcentration. Possibility of stand-off chemical sensing and source localization *via* biorobots.

Odorants binging by biosensor/array of biosensors with suitable coatings (primary transducers) or whole animals integrated with electronics (secondary transducers) with optional supporting materials, *e.g.* CNTs.

Generation of signals from biorecognition elements (peptides, OBPs, ORs, whole animals, *etc.*). Possibility to introduce adaptation layer.

System of collection, processing and data handling. Results visualization.

Depends on the obtained vapour *"fingerprint"* possible warning by software.

Vapours sucked by respiratory system enter canine olfactory system.

Active sniffing (*e.g.* inspire/expire) through the nostrils or "nares", dust and debris filtration.

Inhaled air exposed to the maxilloturbinates where it is warmed, moistened and cleaned by specialized ciliated epithelium and support cells. Odorants selectively bind to ORs and initiate the perception. Ion channels enabling to conduct cations. If the influx of cations increases an action potential is generated that propagates from this OSN along an axon, through the cribriform plate to the glomerulus and mitral cells.

Then on through the olfactory tract and into the brain where the odorant are interpreted. The majority of odorant processing accomplished by the main OE. Generation of olfactory sensations and warning about potential threat.

Fig. 2. Comparison of the principles of operation of canine sense of smell with bioelectronic analogues sensing mechanism. Portions of images reproduced with permission from Refs. [5,24–27].

sampling of the explosives described does not allow the stand-off detection, which is especially needed in military and security areas. There are much fewer reports on explosives biosensors for gas phase analysis as compared to solution analysis methods. Vapour pressure of the explosives varies in a wide range, which constitutes a significant challenge regarding their detection and localization. The aforementioned problems and the concepts of solutions are presented in this review paper. The authors focused on the biomimetic devices directly mimicking the sense of smell and being an inspiration for design of the devices for analysis and localization of explosive and hazardous substances. The materials mimicking biological olfactory systems, such as biomimetic peptides, antibodies, aptamers, molecularly imprinted polymers and non-biological receptor materials employed in the biosensors were recently described by Liu et al. [10] and by Wasilewski and Gębicki [4].

2. Biological olfaction inspirations in electronic devices

The olfactory systems of vertebrates and invertebrates allow detection and discrimination of a wide range of the explosives with low molecular mass, various chemical structure and properties. A vast number of the molecules of gas substances with different structure interact with ORs and are processed by suitable systems such as the brain of mammals or insects. Correct operation of smell in living organisms is indispensable for acquisition of the nutrients and for avoidance of potential dangers. A classic example is common practice from the past when canaries were kept in coal mines as the early warning system against elevated concentration of gases, mainly methane, carbon monoxide and dioxide [4], the substances hazardous to birds and thus to people. The sense of smell, or precisely speaking the chemoreceptors reacting to olfactory stimuli are the most numerous group of membrane receptors and their structure is encoded in the genes of all living organisms. Even in primitive organisms, such as soil nematode, the ability to avoid odours connected with pathogenic bacteria can be observed [16]. All cells are characterized by the ability and necessity to sense the external chemical environment. During the evolution some of them transformed in the cells capable of specialized identification of the external environment, for instance olfactory cells. The olfactory systems belong to conservative systems and some features are common to different types of organisms, vertebrates and invertebrates. It is interesting that convergent evolutionary processes occur between the species, which implies that the olfactory systems are more analogous than homologous in character.

The materials for artificial olfaction devices have not reached their full potential yet as far as detection of the explosives is concerned. Some limitations impede their implementation in favour of classic detection techniques. However, a progress in available materials and their modifications, coupled with suitable transductors can be employed to construction of the sensors and sensor arrays for gas explosives, which was recently described by Wasilewski and Gębicki [4]. Various organic and inorganic materials as well as their composites allow improvement of the sensors' operation. Nevertheless, there is a long way to enhancement of the sensors' efficiency and one of the solutions in this field is utilization of the biomimicking materials and inspiration by the senses of smell present in nature. The concept of biomimetics was defined by ISO as "knowledge gained from the analysis of biological systems to find solutions to problems, create new inventions and innovations, and transfer this knowledge to technical systems" (ISO 18458: 2015). Biomimicry emerged to reconnect design with nature. Unlike bionics and biomimetics, biomimicry is mainly concerned with the invention and implementation of bio-inspired technological approaches [17]. Supramolecular chemistry, a field concerned with the self-assembly of molecules into chemical systems is commonly found in biological architectures and can be applied in design of electronic devices for explosives sensing. Biomimetic strategies in materials research are not just the key to improve conventional chemical processing, they also have a deeper impact on development of artificial olfaction technologies. Application of the biomimicking materials in the biosensors makes it possible to overcome some of the inconveniences connected with employment of animals (sensitivity to saturation, fatigue, distraction, influence of environmental conditions, expensive training) [18]. Moreover, negative influence of the explosives on health of the animals exposed is noted [19], which should be an additional driving force for artificial olfaction development. Better understanding of the fundamental mechanisms of operation of the biological systems is an inspiration to create the biomimicking electronic systems [5,6,20,21]. Biomimicry also allows overcoming some of the limitations of the artificial olfactory systems (low selectivity, specificity) *via* implementation of the biological recognition systems mimicking directly the olfactory systems present in nature [22]. Current trend is to enhance and improve biosensors and their arrays in odour identification and classification abilities by merging with biomolecular recognition systems [15]. Electronic sensor arrays can detect diverse molecules, however the specification of the composition of VOC mixture is challenging and require to use chemometric techniques, which precisely mimics the mechanism of the human brain through large, multi-layer neural network [23]. Comparison of the main operation principles between canine olfaction and bioelectronic analogues is presented in Fig. 2.

2.1. *Mammalians-inspired strategies*

Olfaction enables most mammalian species to detect and discriminate large numbers of odorants. ORs are determined by a large number of olfactory genes that display a high level of polymorphism and nonfunctional pseudogenes [28]. OR genes are the largest gene repertoire in the mammalian genome, representing 2−5% of all protein coding among species [29]. There has been a long-term effort to compare smell sensations in humans and animals. The comparison may develop scientific evidence concerning hypotheses about relative olfactory powers in humans and other mammalian species. An important criterion is the integration of human psychophysical results with animal results in similar studies, as animal results may approximate the neural mechanism and olfactory perception in humans [28].

Development of the techniques for identification of the explosives is an important task from the standpoint of global security of civilians and defence. Detection of the explosives or their precursors, which are characterized by very low vapour pressure, is especially challenging. Trained animals are one of the most efficient methods of explosives identification [30]. Due to the unique operation parameters of the biological olfactory systems, including those of animals, they are still employed for detection of drugs and hazardous substances [10]. Sniffer dogs are one of the most effective methods of explosives detection [31]. Effectiveness of the detection methods for volatile substances is compared with the detection using trained dogs, although other mammals are similarly useful [10]. Special attention should be paid to African elephants, *Loxodonta africana,* which have more than double the number of genes associated with olfactory reception compared with dogs: about 2000 versus dogs' 811. This suggests that olfaction plays an enormous role in elephants' lives and after training makes them reliable TNT detectors [32] with sensitivity and stability greater then canine, even when in the presence of highly volatile distractor odours. Also, U.S. Navy deployed trained dolphins to trace sea-mines [33]. It suggest significant potential of the mammals in sensory analysis and construction of the biosensors [10]. Due to high sensitivity of the biological olfactory systems and confirmed utility of this type of solutions, the receptor materials were elaborated, which mimic unique receptor abilities of biological patterns, biologically-derived recognition elements or biohybrids [34].

The olfactory systems of all mammals intermediate in perception of volatile chemical substances and allow acquisition of the information about the environment, including this about potential hazards, e.g. explosives. A general principle of most olfactory systems is based on adsorption and diffusion through olfactory mucosa, by selective binding of the odorants and transfer using OBPs, and transmission *via* sensory neurons to proper processing centre [35]. The mammalian olfactory systems are based on a large family of transmembrane proteins of ORs, representing the biggest family of genes in a genome of the vertebrates (ranging from around 400 functional receptors in humans to around 2000 in elephants) [32]. Despite a wide range of receptor proteins, the operation mode is similar. Volatile molecules activate common, cAMP dependent, intracellular signal path, open ionic channels and generate an action potential resulting in an olfactory response.

Schematic representation of human olfactory system is presented in Fig. 3.

Diversity of the olfactory systems determines development and elaboration of the biosensors with high sensitivity, which can operate in the stand-off mode. Moreover, application of the components of olfactory systems in the biosensors is not limited to detection of the analytes in gas phase. In practice, the ORs of mammals are present in cilia of sensory neurons, which are embedded in mucous layer inside nose and then the odorants reach to actual olfactory system by diffusion and indirect transport *via* OBPs [35]. In mammals, ORs are mainly located in the olfactory epithelium (OE), where they perform odour detection [29]. Since their original discovery in the nasal cavity, ORs have been found in various other cell types throughout the body where they might regulate physiological cell functions beyond olfaction. Indeed, an increasing number of studies have uncovered ectopic expression of ORs in a variety of organs, including prostate, tongue, heart, testis, brain, gut, hair, skin, etc. Al-Maskari et al. [27] take inspiration from neural circuits of the vertebrate olfactory system and proposed Artificial Olfactory Receptor Cells Model (AORCM). Conducted experiments shown the superiority of presented method compared to alternative state of the art techniques. Furthermore, paper by Al-Maskari introduced adaptation trigger mechanism and demonstrated that presented model could significantly improve electronic nose (EN) instruments performance [27].

2.1.1. *Dogs*

Smell is probably the most fundamental sense for dogs because it is optimized for very detailed perception of external stimuli. The abilities of the sense of smell of dogs are utilized by people for, among others, detection of the explosives, drugs, biological hazards, biomarkers of diseases, searching for missing people, etc. [37]. An ability of selective and sensitive perception of volatile substances is the key factor in case of the sense of smell of dogs. Despite a progress in gas analysis techniques, sniffing dogs are still very effective and reliable tool for identification of drugs and explosives. Moreover, the dogs are also trained to increase biosecurity [38].

Overall view of canine olfactory system with biomimetic instruments are presented in Fig. 4.

The detection limits of canine olfactory system varied from tens of ppb to five hundred ppt. High sensitivity to selected substances facilitates effective detection of the compounds already at the level of a few ppt (N-amyl acetate detected by the dogs as 1.14 ppt and TNT in the 500 ppt range), offering their detection in complex gas mixtures [41]. Dogs are capable of detecting such materials in hardly-accessible places (hidden or covered with masking substances). Dogs' ability of figure-background segregation is the key for identification of HMEs, which can be composed of a broad range of substances constituting odorous mixture. Identification of particular components of the mixture by humans is very troublesome and calls for a time-consuming training. Dogs' abilities to react to concentration of gas substances, which humans are not able to detect, are widely used and they lead to a conclusion that the sense of smell in dogs is much better than in humans. When using explosives detection dogs, this means that the canine directly “takes the lead,” and goes where its instincts and training command. The dog's handler must give the canine equal access to all areas and persons. If a canine alerts, it is because the dog senses something potentially hazardous, which is consistent with the

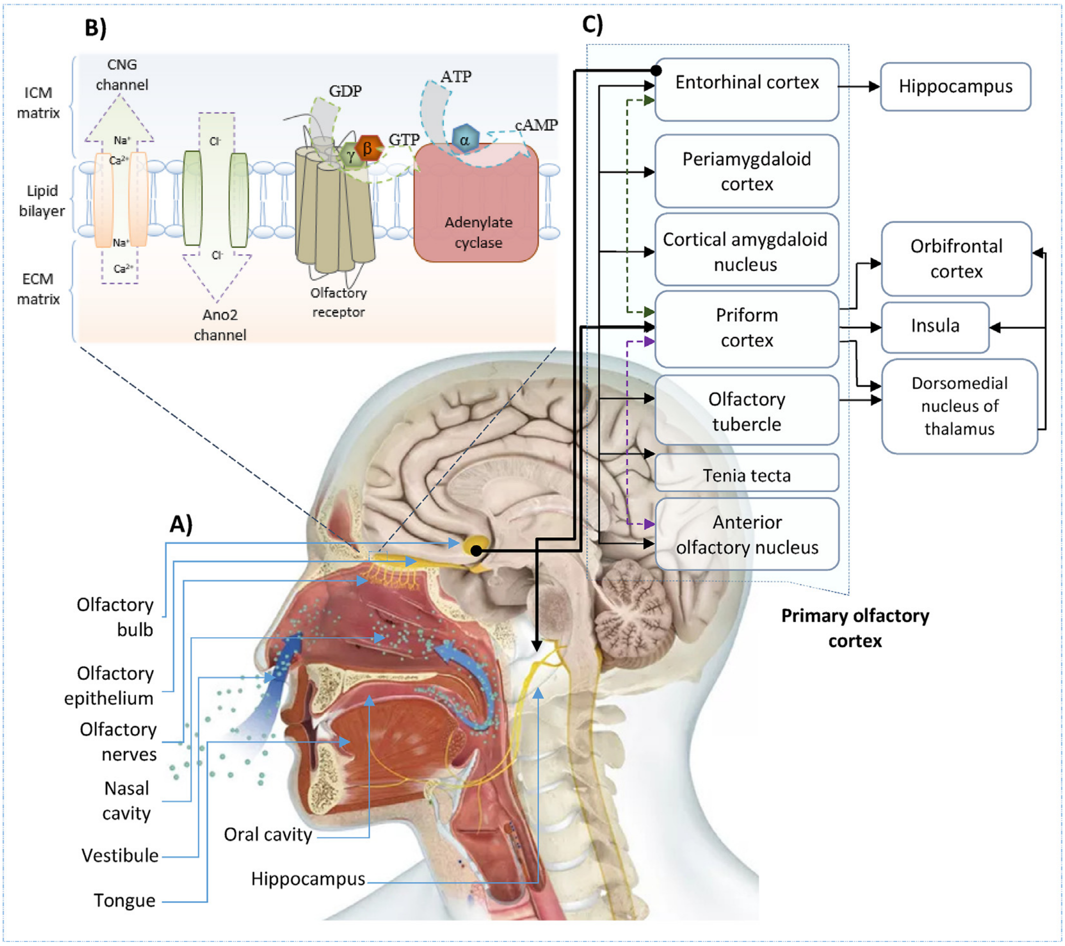


Fig. 3. (A) General scheme of the structure of the human olfactory system located in the facial skeleton (*viscerocranium*). **(B)** Scheme of stucture, generation and signal transduction in the OR. Receptor excitation starts after ligand (odorant) binding and leads to the activation of the membrane enzyme – adenylate cyclase (AC) *via* on of the G-protein subunits (α, β, and γ), it leads to the conversion of GDP into GTP. AC activity leads to an increase in the concentration of cyclic AMP molecules in the cell and the opening of cAMP-dependent cation membrane channels. The opening of ion channels results in the influx of sodium cations into the cell and in consequence in the creation of an action potential in the ON through axon depolarization. A high concentration of Ca^{2+} cations activates chloride channels, enabling the release of Cl^- anions and increase in of the intracellular current intensity. **(C)** The central processing pathways of the human olfactory system with primary olfactory cortical regions and subregions [36].

intent [37]. Dogs can react both to trace amounts (down to ppt level) of a particular chemical compound or to a combinations that make up an odour fingerprint of target materials. There is still a concern to answer is the sense of smell analytic or configural. If it is configural, most animals, including some invertebrates, will recognize complex mixtures as an indivisible entity and an olfactory mixture will smell as a unique odour, rather than as a collection of individual odours kept together [41]. The dogs trained on only a particular constituent of the explosive (the trainer aid) did not respond to the real explosive, suggesting that they had processed the explosives configurally [41]. Nevertheless, big differences in effectiveness of particular dogs can be observed, which is connected with, among others, behavioural and environmental changes, general condition of the guide and dog race [37].

Despite these facts, sniffing dogs are still a gold standard in the field of volatile compounds analysis. However, due to some of the mentioned difficulties, employment of dogs for detection of hazardous and explosive gases is gradually reduced. It must be emphasized that implementation of a dog to service must be preceded by expensive training (≈50 000 USD/dog) and not all dogs complete the training with success. Additionally, dogs' service provides efficiency of ca. 90% in controlled conditions and 50% in real conditions, with the possibility of additional, time-consuming improvement of identification effectiveness [42].

Due to a number of inconveniences associated with employment of dogs for analysis of the explosives as well as limited number of trained dogs available, the devices directly or indirectly mimicking the sense of smell are designed. The devices, which play the role of the sense of smell of dogs are elaborated. Onodera et al. [43] described the perspective of how to establish the function of dog noses artificially for the detection of hazardous compounds, including explosives. Also, presented systems based on the surface plasmon resonance (SPR) biosensors, which utilize antigen-antibody interaction and the method of hazardous samples collection [43]. The Authors identified choice of a selective antibody as the key factor in design of such systems. They used the commercial antibody to compare newly modified sensor surfaces for detection of TNT. Also, the perspective of how to establish the function of dog noses artificially for the detection of hazardous compounds, mainly explosives was described. Park et al. [40] prepared nanovesicles including canine ORs (cfOR5269), specific receptors for hexanal, and fixed them on a carbon nanotube (CNT) channel in a CNT transistor to build an olfactory-nanovesicle-fused carbon-nanotube-transistor biosensor (Fig. 4.3). Interesting and growing concept is to combine canine and digital noses. The potential use of sniffer dogs as mobile bio-detection tools has recently been highlighted [18]. So called "bio-detection" dogs could be trained in identifying specific targets, thus potentially serving as a

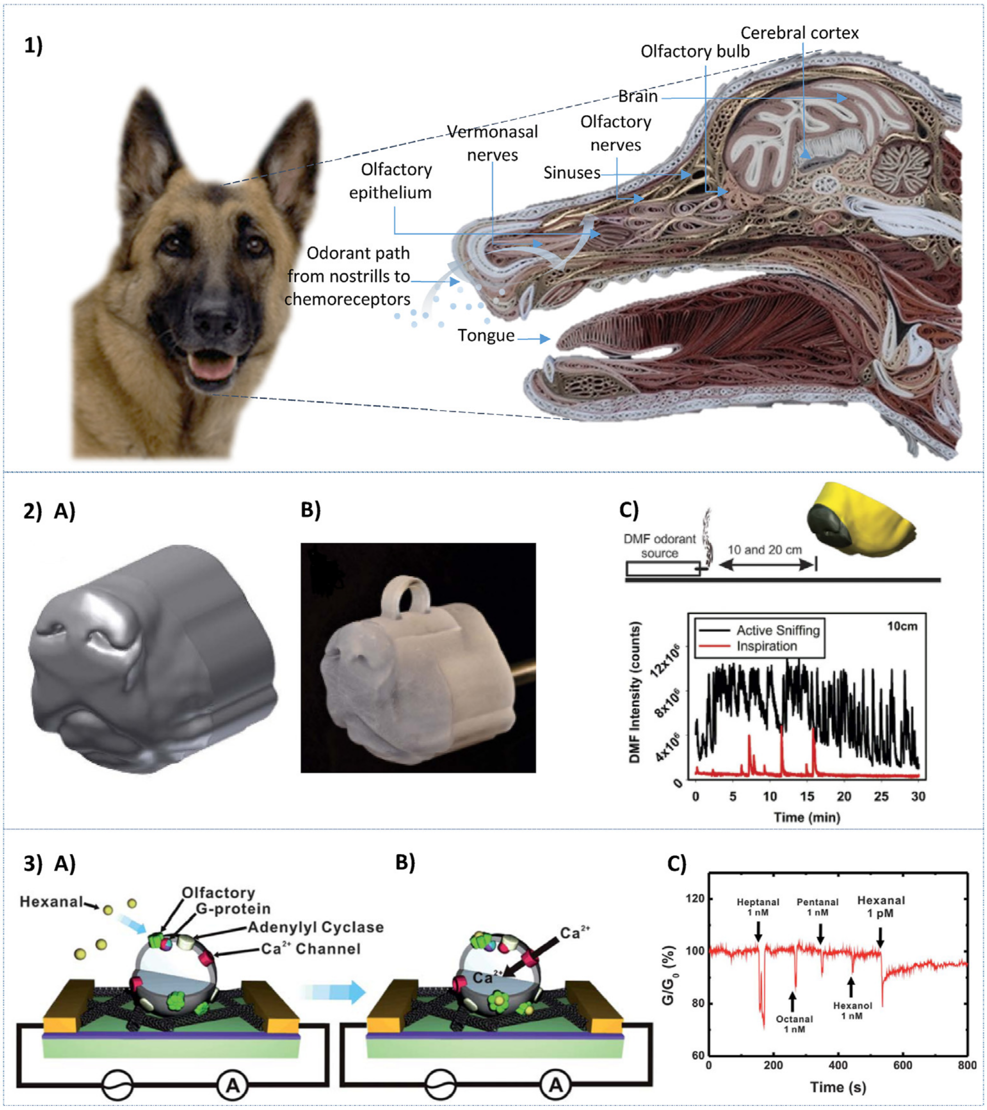


Fig. 4. The canine olfactory system with schematic diagram of a dog head. Axons from the main EO project to the main olfactory bulb (OB), while axons of sensory neurons in the vomeronasal organ project to the accessory OB. **2)** Biomimetic sniffing with 3D Printed Nose of a Dog. **(A)** Reconstructed model of the canine nose based on the model of Craven et al. [39]. **(B)** Photograph of the 3D printed nose that includes a removable polyurethane foam inserted within the flow path in the vestibule of the nose that collects inspired DNT vapour. **(C)** Sampling of DMF vapour by the 3D printed dog's nose during active sniffing with representative signal responses from the mass spectrometer comparing DMF signal intensity at 10 cm for active sniffing versus steady inspiration. Reprinted with permission from Ref. [24]. **3)** Schematic diagram showing the sensing mechanism with an olfactory-nanovesicle-fused carbon-nanotube-transistor biosensor (OCB). **(A)** A CNT-based transistor was coated with poly-D-lysine for the stable adsorption of canine olfactory-based nanovesicles. Then, olfactory nanovesicles were immobilized on a CNT channel region in the transistor. **(B)** The binding of odorants to ORs results in a Ca^{2+} influx into the nanovesicles through Ca^{2+} channels. Here, the accumulated Ca^{2+} ions inside the nanovesicles create a positive gate-potential in the vicinity of underlying CNTs, and the increased potential results in the decrease of conductance in the CNT channel. Response curve of OCBs (n = 5) to hexanal with different concentrations (reproduced with permission from Ref. [40]).

highly accurate and mobile sensory means for rapid diagnosis and in the future even *anti*-bioterrorism weapons [18]. In order to take advantage of high sensitivity/selectivity of the olfactory systems of animals, different biomimetic strategies inspired by the way of gas samples collection were elaborated [44], which can make electronic devices "sniff" like a dog. Dogs active sniffing (e.g. inspire and expire) to acquire an odour sample were investigated by Staymates et al. [24] (Fig. 4).

In summary, the main aims of the studies were focused on:

- Several flow visualization techniques were used to investigate the external aerodynamics of canine olfaction during biomimetic sniffing with a 3D printed dog's nose.
- The vapour sampling performance of the 3D printed nose was evaluated with two chemical detection techniques to quantify the differences between active sniffing and constant inspiration.
- Active sniffing (repetitive inspiration then expiration) increases the amount of analyte drawn towards the dog by almost a factor of 18 in some cases, compared to constant inspiration.
- The development of a bio-inspired inlet on a commercially-available vapour detector created a system that "sniffed" like a dog.
- Measurements with the bio-inspired inlet showed an improvement in analyte detection by a factor of up to 16 while the commercially-available vapour detection system was sniffing, compared to constant inspiration.

- Bio-inspired design principles learned from the dog may be used to improve the performance of next-generation vapour detection technology.

2.1.2. Rodents

Olfactory information is essential for a wide range of rodents behaviours, including navigating, foraging, avoiding predators, kin recognition, bond formation, mate selection, sexual and parental behaviours. Precise identification and evaluation of smell sensations are the key for proper interpretation of behaviour, especially regarding safety to provide survival [45]. Rats and mice are ideal animal models for biomedical investigations because they exhibit many similarities to humans as far as anatomy and physiology are concerned. Olfaction based behavioural experiments on rodents are important for the investigation of sensory coding, perception, decision making and memory formation [23]. Based on the elaborated models, in the olfactory system of rats olfaction starts from air flow through the dorsomedial parts and then it is directed more peripherally, similarly to human respiratory systems [46]. Likewise, rats, mice, and humans each have approximately 30 000 genes of which approximately 95% are shared by all three species [47]. Trapping of the substances in mucous can be enhanced by change of air flow rate over the OE providing effective adsorption *via* suitable ORs. Identification of gases occurs with ORs localized on the surface of OSNs (Olfactory Sensory Neurons) [48]. OSN expresses only one of the thousands of ORs in rat genome. It is assumed that OR recognizes given chemical structure or molecular property, for example particular polar group or the molecules with defined length of the carbon chain. Hence, OSN can be activated by one particular chemical structure, the structure identified by OR. An odorant can, because it often contains several chemical structures, be detected by several ORs and thus several different types of OSNs embedded in the OE. Each OSN expresses one type of G-protein coupled odorant receptor, and OSNs expressing a particular OR project their axons to two glomeruli in each OB. Aliphatic and aromatic explosive compounds displaying different functional groups can be detected by the OSNs located in the rat nasal mucosa [49]. 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 [45]. Individual repertoire of ORs influences on the olfactory information acquired from the environment by OSNs. Comparison of human and mice ORs shows that both species have many common subfamilies. However, mice subfamilies are generally larger than human ones. It suggests that human and mice recognize structurally the same numerous odours but mice can be more effective in their discrimination and identification [50]. Rokni and colleagues [51], recently demonstrated that mice could identify the presence of a target odorant in up to 14 component mixtures when the components were presented simultaneously. They trained the mice using highly variable backgrounds that changed from trial to trial. This rich odour mixture training is likely important for the mice to perform this figure-background segregation. However, this was not explicitly tested by Rokni et al., because they did not include a group receiving a different form of training as a comparison. Furthermore, it is not clear whether performance would generalize to the mixtures containing new components that were not used during training. They demonstrate the possibility of figure-background segregation in rodents. Perhaps similar processes extend to olfactory processes in dogs. This would have important implications for detection dogs and suggest the ability to perform an elemental separation likely depends on training history [52]. Identification of the ligands of particular ORs is difficult but seems to be key from the standpoint of recognition of the olfaction mechanisms and their implementation in the electronic biomimicking devices. General presentation of olfactory system in rodents with biomimicking devices is resented in Fig. 5.

Direct application of rodents for the detection of explosives and hazards is still frequent. Because of rodent's size and costs they can be useful in situations where dogs are unavailable or inappropriate. Using computer-controlled instrumentation, it's possible to train large numbers of rodents in parallel and in an automated fashion [55]. The idea of using rodents to detect and locate vapours has been studied extensively [56]. For example, giant African pouched rats are perfect candidates for discovering hidden landmines by sniffing out the explosive TNT [57]. Suitable training of rodents (mice) also allows their utilization for localization of odour sources [58]. Rodents can be trained to discreetly sniff at security checkpoint and give signal when they detect a threat. Similarly to dogs active sniffing (described in the previous chapter), rodent's way of sniffing is also under investigation [59]. Recently, Reisert et al. [60] discussed the advantages and disadvantages of different methods to record breathing patterns based on mice. They demonstrated that breathing signals recorded using telemetry could be recorded during execution of an odour-guided behavioural task. Rodent offers unique advantages in developing biologic odour detection systems. He et al. reported apparatus designed to train maximum 5 mice automatically to detect odours using a new olfactory, relative go no-go, operant conditioning paradigm [53] (Fig. 5.2). Presented results suggest that this odour detection method is promising for further development in respect to various types of odour detection applications. Different biological components of the rodents' olfactory systems are employed as the receptor elements in the biosensors [14]. The biosensors based on OSNs are characterized by unique specificity, sensitivity and response time [61]. However, there are certain limitations in their construction, utilization and effective operation, including short lifetime and difficulties in isolation from the olfactory system. Moreover, they undergo biodegradation in the *in vitro* environment. Accordingly, lifetime and recovery of the biosensors are limited. An important contribution is the paper of Gao et al., who presented the design, construction and test of an *in vivo* biomimetic olfactory system for specific detection of TNT [26] (Figs. 5.1 and 7.4). In this study, they utilized the genetically labelled murine M72 OSNs with the green fluorescent protein (GFP) as the 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 [26]. The electrophysiological responses showed high reliability, reproducibility and specificity for odour detection, and particularly, the high sensitivity to the detection of the odorants that contain benzene rings. Furthermore, presented results indicated that constructed system provided limit of detection (LOD) for TNT in liquids at the level as low as 10^{-5} M and could distinguish TNT from other chemicals with a similar structure. Thus, study by Gao et al. demonstrated that the *in vivo* biomimetic olfactory system could provide novel approaches to enhancing the specificity and increasing working lifespan of the olfactory biosensors, showing their great potential in explosives detection. Preechaburana and colleagues have proposed an SPR optical system on cell phones. The optical system was made with polydimethylsiloxane (PDMS) rubber [62]. The cell phone's display and camera were utilized as a light source and a detector for SPR monitoring, respectively. Mouse anti-human β 2 microglobulin monoclonal antibody immobilized on Biacore sensor chip CM5 was set on the optical system for a demonstration. Interactions with 1.32 μg/mL and 0.132 μg/mL β2 microglobulin were observed on the system. TATP is an easily obtainable explosive compared to TNT and RDX. Therefore, detection methods for TATP are highly required.

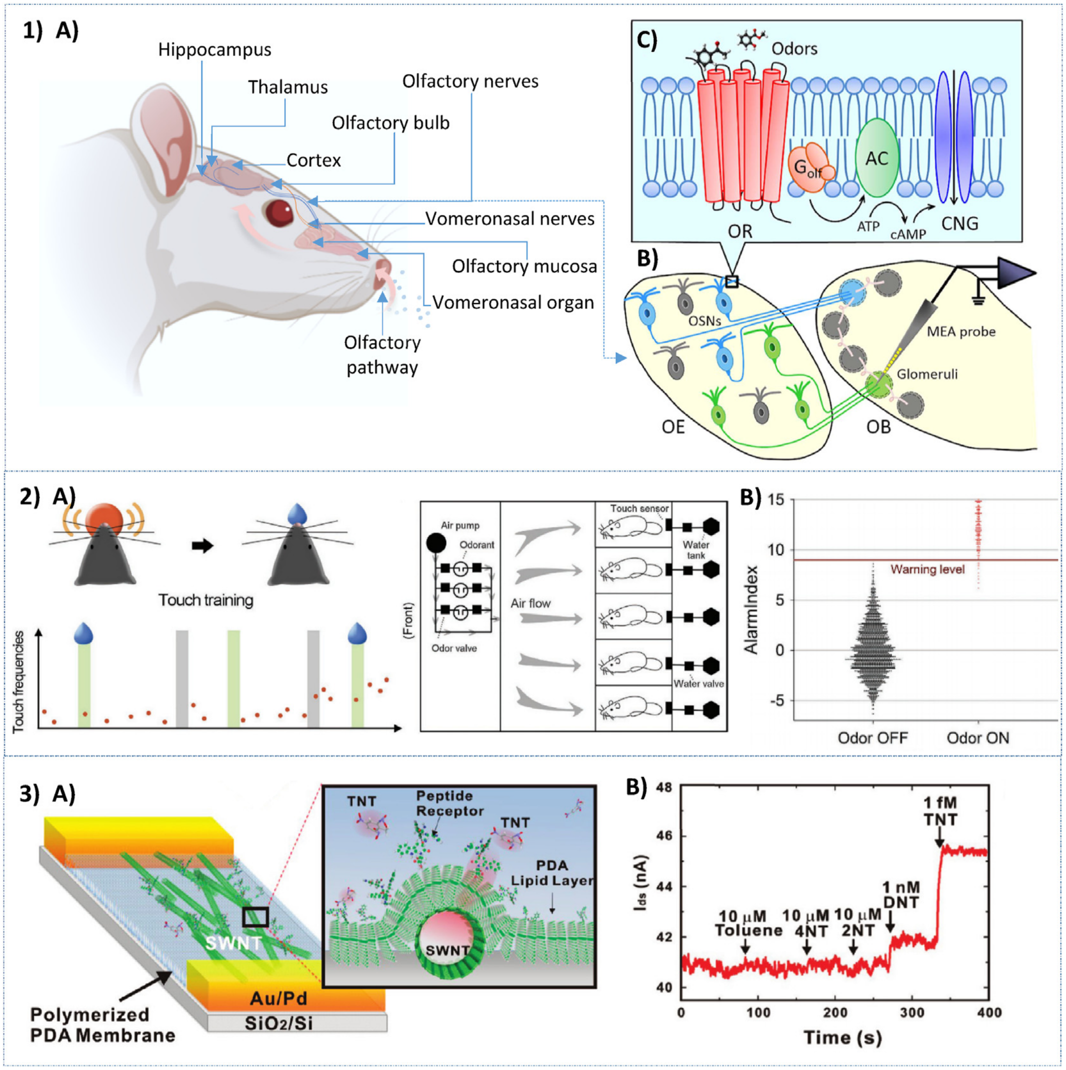


Fig. 5. Olfactory system in rodents. **(A)** Scheme of the main olfactory structures in mice. **(B)** Scheme of *in vivo* electrophysiological recordings from M72 OSNs by implanted MEA. **(C)** Mechanism of signal transduction. The G protein/cAMP pathway underlies signal transduction in the OSNs. Reproduced with permission from Ref. [26]. **2)** An odour detection system based on automatically trained mice by relative go no-go olfactory operant conditioning. **(A)** Abridged general view of the training procedure. **(B)** Dot histogram of AlarmIndex across 4 blocks of the test. Reproduced with permission from Ref. [53]. **3)** Selective and Sensitive TNT Sensors Using Biomimetic Polydiacetylene-Coated CNT-FETs. **(A)** Schematic representation of peptide receptor-functionalized SWNT FET sensor. **(B)** Selective response to TNT in mixed solution of toluene, 4NT, 2NT, and DNT. Reproduced with permission form [54].

Walter et al. developed a TATP analogue to synthesize the immunogen for generating antibodies in two mice. LOD for TATP in ELISA using sera obtained from immunized mice were 65 ng/mL and 870 ng/mL, respectively [76]. Fukutani et al. developed a new type of a yeast-based biomimetic odour sensor [63]. In that study, the replacement of the N-terminal region of the mouse olfactory receptor OR226 with the corresponding regions of the rat I7 receptor mOR226 affected the expression and localization of the receptor and improved the sensing ability of the yeast cells for DNT. Their strategy has a high potential for establishment of an odour sensor system with OR-expressing yeast, elevating the odorant-sensing. Another approach for environmental analysis was performed using biomimetic polydiacetylene-coated CNT-FET. The SWNT-FET sensor device interfaced with PDA-based lipid membranes coupled with TNT receptors and was exploited to transduce the binding activities between the target TNT and its selective peptide receptors [54] (Fig. 5.3). Similar approach was exploited by Kim et al. [54]. The resulting nanovesicles possessing the diacetylene π-electron-conjugated backbones transduce the binding activity of the TNT target molecules by exhibiting specific colour changes upon exposure to the TNT molecules. In the nanovesicle-based biosensors, interaction between odorants and ORs triggers cell signal pathways, and leads to a charge accumulation, thereby allowing sensitivity amplification in signal transduction.

2.2. *Insects-inspired strategies*

Insects are known for their unique olfactory properties enabling detection of trace amounts of volatile substances from very long distance, which is of fundamental importance as far as survival, reproduction and communication are concerned [64]. Delahunt et al. focused their investigation on the sense of smell of insects [64], which is regarded as a simple neuron system capable of learning. Its structural features process the olfactory stimuli *via* a cascade of networks, inside which significant shifts of dimensions occur after each stage where rarity and randomness play a key role in odour encoding. They constructed a computational model of the *Manduca sexta* moth olfactory system that was able to robustly learn new odours, where simulations of integrate-and-fire neurons match the statistical features of *in-vivo* firing rate data. The olfactory systems of insects are based on odour detection *via* trapping of the molecules by extracellular proteins and membrane-bound OR [65]. In case of the insects selectivity and sensitivity of the olfactory systems are achieved due to presence of tiny soluble OBPs [35]. High concentration of these proteins in nasal secretion of the vertebrates suggests high significance in perception of odorous substances and substantial contribution to ligands binding. Both OBPs and ORs contribute to specific cell response and lead to high selectivity of olfaction. OBPs act as the ligands selectors and transporters, initiating a cascade of olfactory signal transduction *via* binding of small hydrophobic molecules aimed at increasing their solubility in water and transport to suitable ORs in a cell membrane. In insects, the early chemoreception steps involving primary contact with chemical signals and the activation of signaling pathways. They occur in porous chemosensory hairs. Two ORs are necessary for activation – a specific receptor and a common receptor. Odorant binding triggers a signal transduction cascade that results in neuronal firing and brain-centered perception of the odorant, which releases the appropriate behavioural response [66] (Fig. 6.1). The cascade transmission of the olfactory signal is accompanied by generation of the nerve impulses, which are ultimately directed to brain where they undergo processing [67]. Huge variety and relatively high stability of some OBPs cause that they are often employed to construction of the odorant biosensors [68]. OBPs belong to the key elements of the chemosensory systems and they possess high potential in development of the receptor elements for the biosensors for detection of natural and synthetic VOCs [35]. Moreover, OBPs not only can be expressed and purified easily, but also are highly stable to temperature (>100 °C), pH, solvents and proteases [68]. It must be mentioned that their utility in the bioelectronic systems has just started to be developed [68]. Methodological and equipment approaches based on synthetic counterparts mimicking activity of the ligand binding sites, which occur in OBPs and ORs are under constant elaboration. Potential application of the elements of the olfactory systems of insects in the biosensors is mainly connected with the challenges related to conformational behaviour involved in the activation of this ion channel, efficiently transducing a binding event into an electronic signal and retaining the binding affinity while the receptor operates outside its natural environment [69].

One of the first examples of insect's utility is employment of honeybee to the olfactory investigations in order to bring closer the behavioural and neurobiological fundamentals of the sense of smell of insects. Unique abilities of the insects to identify chemical signatures encouraged scientists to train bees at detection of the explosives and other analytes [72]. Honeybees can be trained at identification of the explosives such as TNT, C4, TATP at the ppt level. Similarly to rodents, the cost of short training (several days) is low; large populations and capability of fast coverage of analysed area by the insects make them suitable candidates for analysis of the explosives [73]. With Pavlovian conditioning, honeybees can be trained to respond to TNT, Semtex, PE-4 and C-4 down to the detection limits of 78 ppt [72]. The EU-funded ‘Biological Method (Bees) for Explosive Detection’ project utilizes trained bee colonies to identify landmines. It is accomplished with active and passive methods where the first one takes advantage of artificial intelligence and drones to follow the bee colonies soaring above the mines. Also, free-range honeybees can electrostatically collect particles from the air in the regions of their activity, which upon combination with organic foils detecting vapours of the explosives, placed at the hive's entrance can serve as a passive system of explosives detection [72] (Fig. 6.2). Preconcentration of target molecules onto a substrate can provide a method to collect higher amounts of analyte for analysis. The preconcentrates used by Gillanders et al. [70] (Fig. 6.2), placed at the entrance to a hive, are an interesting alternative for field detection of the explosives in real time and they can significantly improve the methods of sampling of the explosives present at the trace level.

Due to unique properties of the biological sense of insects, their subsystems can used in the biosensor technology. Insect odorant receptors are believed to be a complex of an odorant binding subunit, OrX, and an ion channel forming subunit, OR coreceptor (Orco) [74,75]. Construction of a biohybrid odorant biosensor using biological OR with Orco embedded into bilayer lipid membrane on a chip was recently presented by Misawa et al. [74]. To obtain OR (OR8) and Orco (OR7) proteins of yellow fever mosquito (*Aedes aegypti*), they used a reconstituted cell-free translation system derived from *Escherichia coli* (*E. coli*). In addition, sensor integration with a mobile robot was tested with successful demonstration of robot movements triggered by gas stimuli. Khadka et al. have shown that Orco and OrX/Orco coupled biosensor devices can sensitively and selectively detect their target ligands down to subfemtomolar levels using electrochemical impedance spectroscopy (EIS) [76,77]. Peptide sequences derived from OBPs of *Apis mellifera*, have been used to detect TNT [78] (Fig. 7.1). Also, nanotube encapsulated by bombolitin II, a variant of a bumblebee venom-derived amphiphilic peptide, also showed selective molecular recognition of trinitrotoluene among nitroaromatics [79]. Utilization of the protein components, such as the regions specific for particular ligands, or synthetic peptides belongs to new trends in volatile compounds analysis, including the explosives. Researchers have spared no effort to make great progress to further facilitate and improve the properties and to broaden the range of applications of this class of biomimetic [14,80].

3. Biomimetic materials

By mimicking biological senses of smell it's possible to create sequence-specific biopolymers to generate highly selective receptors for explosive compounds [81]. The diversity of chemistry and structure produced by sequence-specific biomimetic recognition components as high-affinity aptamers, antibodies, MIPs and peptides allows specific multivalent receptors to be created for a wide range of target ligands [10].

Aptamers are extensively used as recognition components for biosensor construction, which offer a promising alternative for cheaper, quicker, and simpler detection [82]. Most of the aptasensors developed are based on tagged aptamers [83]. The recent aptamer-based bioassays for explosives were listed by Liu et al. [10]. Moreover, peptide-, antibody- and MIPs-based bioassays were comprehensively presented. To tailor peptides for binding target ligands, several engineering techniques may be applied, e.g. peptide rational design, phage display, computational screening. Presented techniques broadening peptide sequence variability and helps to find sequences for specific VOCs with tunable selectivity/sensitivity. Recently, three TNT binding candidate peptides have been rationally designed and derived from the anti-TNT monoclonal anti-body through the amino acid sequence by Wang et al. The results obtained by using SPR transduction, demonstrated that peptide TNTHCDR3 was determined as TNT binding peptide and no non-specific binding was observed [84,85]. A NIR optical biosensor based on peptide functionalized SWCNTs hybrids for 2,4, TNT detection was developed by Wang [86] as a promising instrument for security applications. A highly sensitive and selective DNT gas sensor using DNT-specific binding peptide functionalized rGO was demonstrated by Lee et al. [87]. Additionally, this device platform was able to provide reproducibility and a regenerated surface for utilization in real field applications. Identification of trace amounts

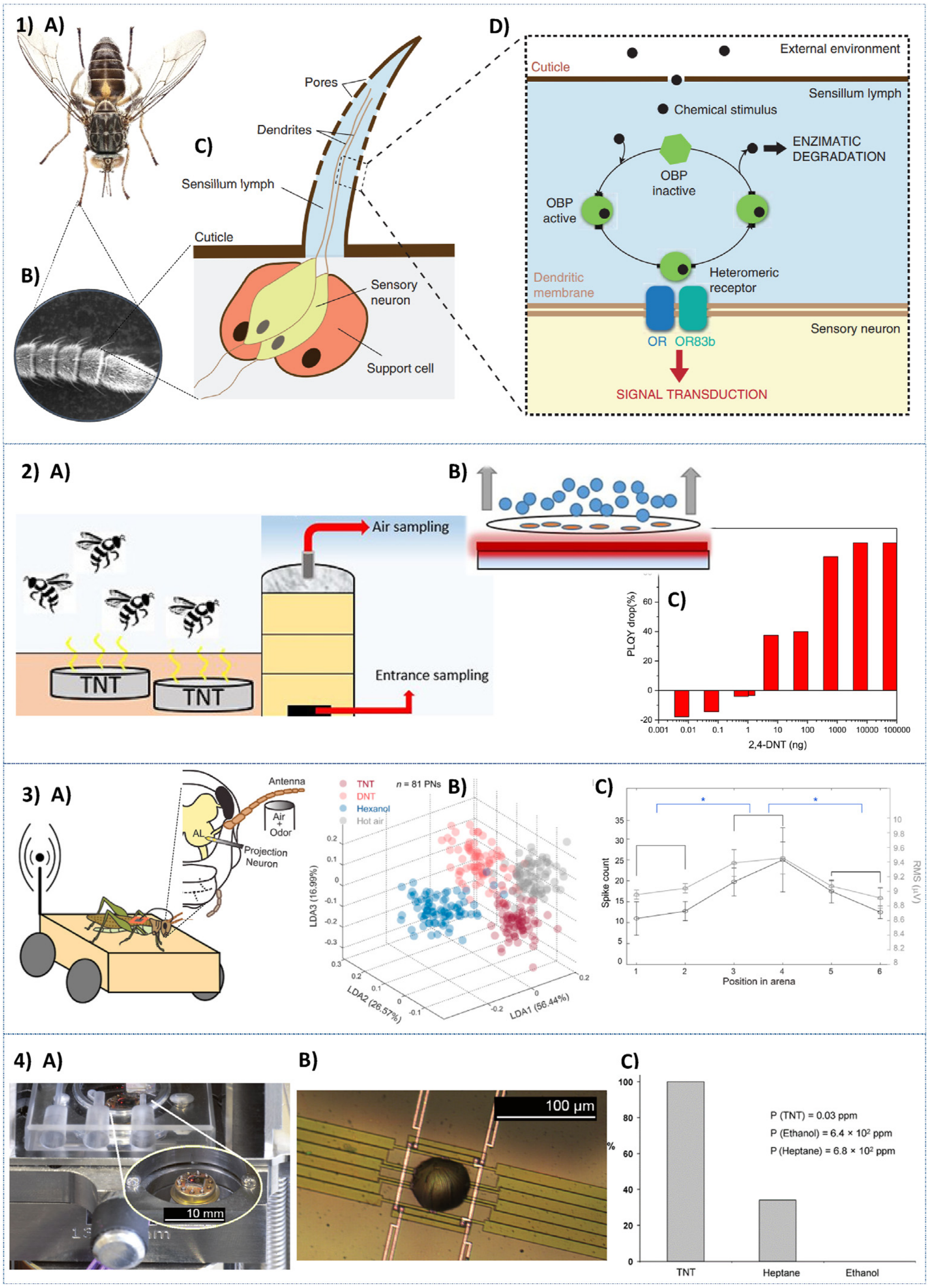


Fig. 6. Schematic representation of insect olfaction. **(A)** Full view of Tsetse fly (*Glossina* spp.). **(B)** Magnification of antennae with sensilla (SEM image). **(C)** General structure of insect olfactory hair. **(D)** The first molecular steps (perireceptor events) of the insect chemosensory signaling transduction pathway. Reproduced with permission from Ref. [66]. **2)** Scheme of the preconcentrator at the hive entrance for collecting explosive materials. **(A)** Collection of explosives by free-flying honeybees in contaminated field. Colony entrance and/or air sampled with preconcentrator filters. **(B)** Thermal desorption of explosives vapours. **(C)** Determination of the LOD of the Super Yellow film to 2,4-DNT molecules in acetonitrile solution. Reproduced with permission from Ref. [70]. **3)** Explosive sensing with insect-based biorobots. **(A)** A locust is implanted with electrodes in its olfactory circuits and placed on a movable car, electrical signal is transmitted wirelessly in real time. **(B)** Responses of 81 projection neurons during odour presentation window (visualized after linear discriminant analysis. **(C)** Spiking activity obtained from the locust is shown as a function of position in the arena. Reproduced with permission from Ref. [25]. **4)** Bio-inspired nanostructured sensor for the detection of ultralow concentrations of explosives. **(A)** Nanocalorimetric chip placed in the AFM with and without the cantilever above the chip. **(B)** Hemispheric TNT particle loaded on the heatable membrane of a nanocalorimeter to generate pulses of ultra-low concentrations of the explosive vapour. **(C)** Relative responses of the cantilever covered with TiO_2-NTs to different vapours. Reproduced with permission from Ref. [71].

of the explosives is one of many tasks, which can be facilitated thanks to employment of the insects [70]. Some researchers have taken advantage of MIP integrated with AuNPs for TNT detection for enhancing sensing parameters [88]. MIPs are promising biomimetic coatings, which can be used in a wide range of conditions; they are relatively stable, simple to produce, and easy to modify for a variety of target molecules [89]. However, novel synthesis methods [90] and sampling methodologies (e.g., spraying micro-size organic droplets which are loaded with MIP nanoparticles into the gas samples [91]) are required to overcome limitations

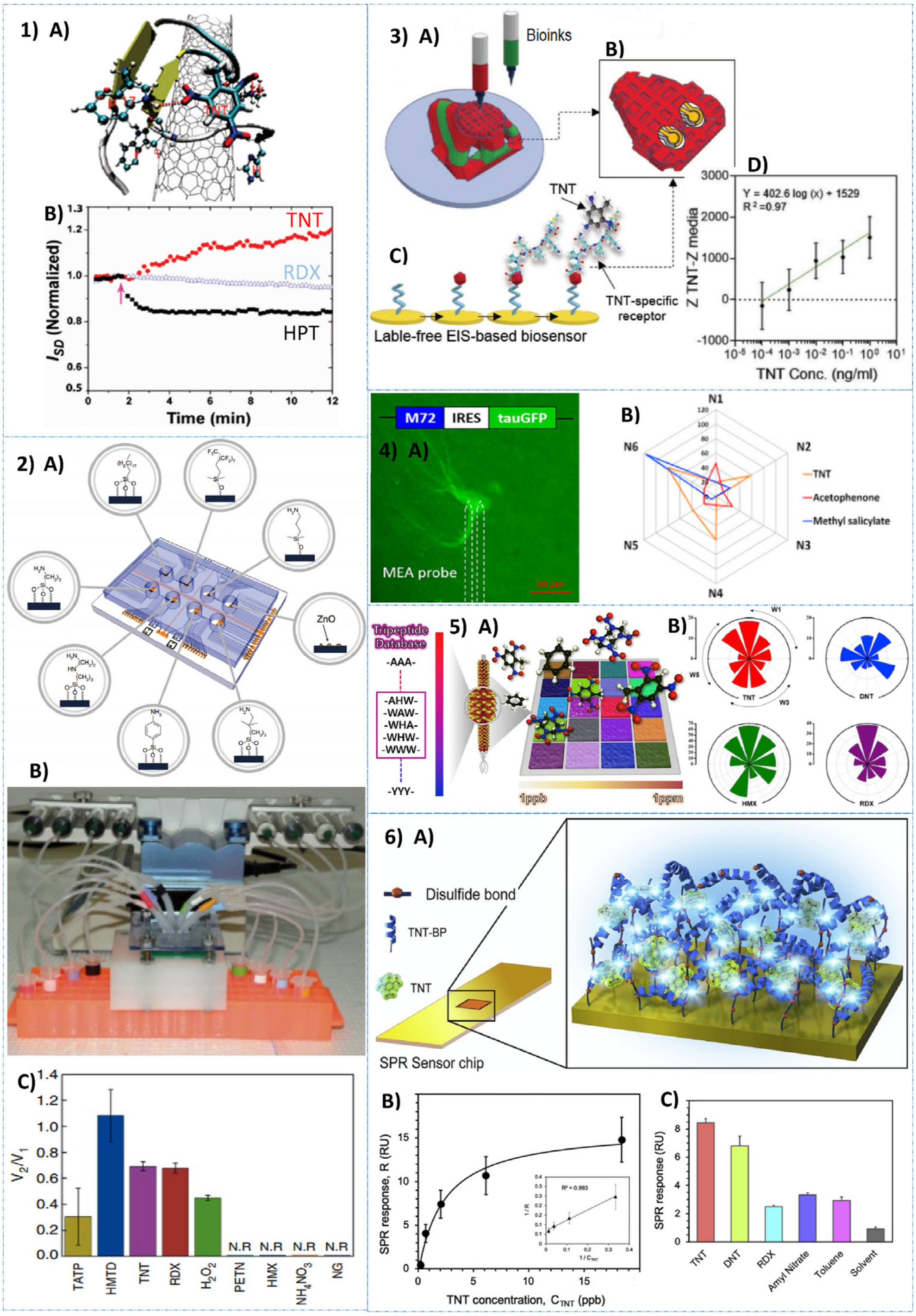


Fig. 7. Examples of B-EN systems for explosives detection. **1)** SWNT-FET sensor functionalized with peptide recognition element **(A)** predicted with molecular dynamics simulations immobilized onto the SWNT surface. **(B)** Sensors responses to TNT, RDX, and HPT. Reproduced with permission from Ref. [78]. **2)** NanoFet-based multiplex sensing chip with **(A)** eight chemically differentiated subregions **(B)** connected with fluidic device. **(C)** Discrimination of explosive compounds through their respective kinetic ratios Reproduced with permission from Ref. [122]. **3)** A 3D-printed hybrid nasal cartilage with functional electronic olfaction. **(3)** 3D-printed hybrid bionic nose. **(A)** Tunable dual bioink system. **(B)** Biosensing mechanism based on label-free EC EIS for TNT detection **(C)**. **(D)** Calibration curve for TNT sensing using cell culture media. Reproduced with permission from Ref. [123]. **4)** In vivo B-EN for TNT detection. **(A)** Scheme of in vivo OSNs recordings via MEA probes with photomicrograph of Pt black modified probe tip with section of OB containing mature M72-GFP glomerulus. **(B)** Polar plot of average responses of six neurons to odours. Reproduced with permission from Ref. [26]. **5)** Optical B-EN of outstanding sensitivity and selectivity toward volatile organic compounds implemented with genetically engineered bacteriophage. **(A)** General scheme of highly functional biosensor design and preparation. **(B)** The fingerprint patterns of phage-based sensor arrays when exposed to 100 ppb explosives. Reproduced with permission from Ref. [124]. **6)** The scheme of bioinspired TNT-BP matrix for the detection of TNT. **(A)** Cys- TNT-BP was applied to SPR via disulfide bond formation. **(B)** Relative SPR response of the TNT-BP matrix. **(C)** SPR response to different molecules at 100 nM concentrations. Reproduced with permission from Ref. [115].

with employment in gas analysis and successful commercialization. Electrochemistry has played an essential role in developing fabrication methods for biological processes and biosensors offering a simple and inexpensive approach for rapid and on-site monitoring of explosives. Despite great success, in comparison to biological olfaction, classic chemical sensors are often lack of the specificity and selectivity for the target explosives [4], by utilizing biological and biomimetic coatings it's possible to enhanced (bio)sensors metrological parameters [10]. Moreover, the explosion of activity in nanoparticles and nanotechnology and its huge potential has dramatically affected the biosensor technology, opening novel paths of research for the electrode components and signal transduction from biomimetic layers [92]. Different kinds of nanomaterials are available for immobilization, including gold, silver, silicon, and copper nanoparticles. Meanwhile, carbon originated materials, like graphite, graphene, and carbon nanotubes, have found application in biosensors on the electrode surface and facilitate sensitivity, specificity, conductivity, and selectivity [82]. The specific binding to one of the most recognizable explosive molecule – TNT, by biosensors with comparison to classic analytical techniques and animals is presented in Table 1.

4. Localization of smell

Understanding how the information about volatiles/odorants is coded in the olfactory system, in many practical applications allows utilization of chemical identification with the artificial olfaction techniques and can offer the possibility to establish odorants classification systems [23]. It is also important to understand how the organisms localize an odour source and to establish navigation algorithms. Tracing of the odour sources is commonly employed by vertebrates and invertebrates, which provides survival by localization of food, mate, predators, adequate habitat [58]. In large naturalistic environments, odour plumes become characterized by odour fluctuations, providing animals with a dynamic odour environment to navigate with different behavioural strategies [56]. To systematically determine the strategies that may account for rodents odour-based navigation, scientists have turned to robotics [56]. Inspired by the animal evolution, the biomimetic robots are integrated with their biological recognitions systems, including the sense of smell, which give them more powerful motor abilities, cognitive abilities and more delicate control processes than other robots [99]. Simultaneously, development of the biomimetic technologies and improvement of the interactions robot-animal/animal's detection system contribute to better knowledge about animals' behaviour [100].

A mixture of odorants in air is not a smooth, uniform 'cloud'. In turbulent environment a spectrum of odorants is wide, non-uniform and unstable, with particular dominant scents. In the case of animal, even if it is present close to the odour source, its detection is difficult and occasional. Moreover, local concentration gradients do not usually point at the source during identification [44], which was noticed on the example of moths attracted by the pheromones as they did not fly straight towards the source. Problems connected with the odour-localizing robots were identified below as tasks to face:

- Gas finding detecting an increased concentration of a target gas.
- Gas source tracing following the cues determined from the sensed gas distribution (and eventually using other sensor modalities) toward the source.
- Mapping of gases distribution, tracing of gases upon strong, constant air flow and in its absence.
- Gas source declaration determining the certainty that the source has been found, including full gas source localization based on analytical models.

There is significant interest in design of the robots capable of searching and identifying the odour sources, for instance to trace the odours emitted by drugs, chemical leaks, explosives, etc. [4,99]. Implementation of the biomimetic algorithms integrating the calculation models of the main anatomic structural blocks of the olfactory paths engulfing operation of OB and olfactory cortex of the vertebrates or the antennal lobe and mushroom bodies in the insects was presented by Persaud [3]. These have been tested with an odour robot with navigation capabilities in mixed chemical

Table 1
Examples of TNT detection techniques [10].

Detection	Recognition principle/ element	Response time	LOD	Ref.	Advantages	Disadvantages
Antibody-based	Anti-TNT PcAb	5 s	0.22 mM	[93]	High affinity and specificity; Limited sample treatment; Rapid detection	Difficulties in fabrication; Non-specific adsorptions; Risk of the favored dissociation; Low thermal stability
Peptide-based	SPR, ARGYSSFIYWFFDF, TNTHCDR3	12 s	15 μM	[84]	Rapid, label-free detection; Good stability and reproducibility; Can be easily modified, synthesized and purified	Moisture decrease long-term stability; Synthesis limited to specific aminoacids
Aptamer-based	Nucleotide: 126, 118. Enzyme-linked aptamer-sorbent assay	Real-time	1.76 nM	[94]	High affinity and specificity; Flexibility in structure design; Reproducible production	Aggregation problems; Problems with aptamers generation, time-consuming and labor-intensive protocols (e.g. SELEX)
MIPs-based	Poly(carbazole-aniline), CV	Real-time	110 nM	[95]	Cost-effective and simple synthesis of high stable and reproducible polymers; Label-free detection;	Incomplete removal of the template; Random distribution of binding sites, Slow mass transfer; Irregular morphology
MS-based	LVI-GC-MS	~10 min	0.44 pM	[96]	High resolution and molecular identification; Screening of a large number of explosives simultaneously; Reliable analysis;	Trained staff; High costs and complexity; Sample preparation; Limited dynamic range; Complex spectra
IMS-based		100 μs	0.15 ng	[97]	Rugged, easy-to operate; Real time monitoring; High sensitivity; No vacuum pumps; Portability	Matrix effects; Low resolving power; Limited selectivity
Spectroscopy-based	SERS	1 s	29.98 nM	[98]	Highly molecularly specific; Simultaneous detection of multiple explosives	High costs; Specific instruments
Animal-based	Detection dogs	Real-time	ppt/nM level	[41]	Versatile, mobile and real-time detection of traces, even when they are masked	Complicated and expensive training; Obligate handler (usually one-handler-to-one-dog mode); Detection affected by physiological state, surroundings, fatigue

LVI – large volume injection; SERS – surface-enhanced Raman scattering.

plumes. Martinez [101] presented the search algorithm named „infoaxis". This algorithm takes whatever action needed that maximizes the expected reduction in entropy of the source probability field, and therefore the rate of information acquisition controlling movement and direction of movement appropriately. Recently, whole body systems of the insects were included into the robotic systems to control the robot with the signals from intact antenna [102]. Also, insect electroantennogram from excised antennae was proposed by Ando et al. to control robots [103]. Moreover, Martinez et al. [102] incorporated entire olfactory systems of the insects into the robotic systems to control the robots and localize odour *via* intact signals form insect antenna. Recently, Adnerson et al. [104,105] patented bio-hybrid odour-guided autonomous palm-sized air vehicle equipped with the odour-sensing antenna of an insect. They demonstrated that insect antennae respond more quickly than metal oxide gas sensors, enabling odour localization at an improved speed over previous flying robots [105]. Recently, Saha et al. [25] proposed a hybrid device based on the olfactory sensors and sophisticated neural computational framework available in an insect olfactory system (Fig. 6.3). Used mobile multi-unit electrophysiological recording system allowed to construct biorobotic explosive sensing system. They show that selected subsets of neurons in the locust (*Schistocerca americana*) brain were activated upon exposure to various explosive chemical species (such as DNT and TNT). Responses from an ensemble of neurons provided a unique, multivariate fingerprint that allowed discrimination of explosive vapours from non-explosive chemical species and from each other. This chemical sensing approach directly takes advantage of the rich repertoire of sensors in the insect antenna, and the processing features of the olfactory circuits downstream to the sensory neuron [25]. Localization of the source of gases/chemical substance is of substantial importance from the standpoint of explosives identification and it can also be realized with the gas sensors and sensor arrays, which can be places in the mobile devices, such as drones or mobile robots [106,107]. An EN embedded on a mobile robot is a reliable option for implementing tracking algorithms for localization, however understanding the behaviour of EN in real scenarios where external factors may affect the robot performance is a challenge. Different effects of chemical sensing and the possible challenges to overcome in chemical source localization were presented by Trivino et al. [107]. Recent advances in sensors miniaturization and in low-cost small drones are catalysing exponential growth in the use of such platforms for environmental chemical sensing applications [108]. Lessons learned from the vertebrates and invertebrates smelling systems may benefit the next-generation of vapours detectors for explosives and could inform future bio-inspired designs for optimized sampling of odour plumes [24].

5. Bioelectronic analogues

There is a constant research trend in the field of chemical sensing and biosensing to follow physiological principles in the animal senses of taste or olfaction, by using arrays of non-specific receptors merged with accurate chemometric tools. These bio-inspired principles have evolved into analytical systems like ENs (for gas analysis) and ETs (for liquid media) [109]. Despite relatively big commercial success of the EN and ET instruments, mainly in medical diagnostics, food products analysis, they still have some fundamental limitations regarding low specificity and sensitivity to the environmental conditions, which precludes their effective implementation to routine analysis of hazardous and explosive substances [4]. Also, periodic recalibration of EN and ET is required to maintain its accuracy over time. To reduce recalibration effort, calibration transfer methodologies have been proposed in the literature [4]. These limitations motivate to search for an inspiration in nature, thus to construction of the biomimetic devices [20]. Despite obvious challenges, it should be noted that significant progress in ENs is attributed to the interdisciplinary investigations in the field of nanotechnology, biotechnology and electronics. These efforts are propelled by novel nano and micro technologies of fabrication; better understanding of molecular electronics, improved techniques of biofunctionalization and gas sampling. The knowledge of ORs' biochemistry substantially contributed to development of ENs and construction of the devices called bio-electronic noses [14]. Dynamic development of chemical identification techniques resulted in elaboration of the biosensors where the receptor elements directly or indirectly mimic the biological sense of smell. Some solutions follow the concepts from chapter 3.

An inspiration with the olfactory system of mammals resulted in a design of the artificial devices, which combine the arrays of chemical (bio)sensors with suitable pattern recognition systems. The EN and ET devices, thanks to Dodd and Persaud, have been systematically developed since 1982. Due to potential parallelisms with human sense of taste, ETs have been mainly utilized for food and beverage analysis [6,110]. There are only a few examples in recent literature with ET principles adopted to detect and quantify nitro and peroxide explosives [111–113]. Presented by González-Calabuig et al. [111] voltammetric sensor array was used to qualitatively identify different explosive mixtures such as IEDs. The Authors suggested that voltammetric ETs could be of application for the detection of real explosive formulation samples and a good candidate for homeland security applications; leading to a new generation of on-site field deployable explosive detectors [111,114]. Combination of the gas biosensors into arrays yielded the concept of a B-EN [5]. In some aspects the B-EN differs from conventional ENs, where the active material is made of the chemosensitive elements with a wide range of response. Some limitations of ENs stem from impossibility of direct mimicking of the biological olfaction, which is connected with a lack of the bioelements capable of such action. A fundamental assumption in construction of the B-ENs is utilization of the domains of biological origin as the active elements of the biosensors. It allows identification of given odorants independently of physical properties and complexity of sample's matrix. Diversity of the olfactory systems present in nature creates large possibilities of acquisition of the biological elements for construction of the biosensors [14,77]. Apart from typical biological components of the olfactory systems, such as proteins of the ORs, the odorant biosensors also employ the structures mimicking biological materials, such as synthetic peptides and MIPs. Although peptide aptamers are attractive candidates for a molecular recognition because of their ease of synthesis and chemical stability, they still have difficulty in applying to highly sensitive detections [10,115]. Thanks to direct combination with the biological olfaction, B-ENs allow analysis of odorants in complex environmental samples [3,116,117]. Recording and processing of a biological signal from a bioreceptor element can be accomplished with, among others, microelectrode arrays (MEAs), EIS, quartz crystal microbalance (QCM), surface acoustic wave (SAW) bulk acoustic wave (BAW), field effect transistors (FETs), SPR sensors, conducting polymers (e.g. polypyrrole), carbon nanotubes, graphene, etc. A special attention is paid to the QCM and FET-type technologies for application in B-ENs [14,22,118]. The latest achievements in nanotechnology and biotechnology encourage to broaden the application fields of B-ENs Their characteristic feature in sensory analysis is high sensitivity, selectivity and specificity. Analysis of odorants with the biosensors enables analysis in liquid phase at the level of fM and at the ppt level in gas phase [77,119,120], which is close to the LOD of dogs' nose [59,121] (Fig. 1). Fast operation, non-invasive sample analysis and ease of measurement make ENs a real alternative to other popular methods of sensory analysis [106]. Moreover, the possibility to more precisely mimic human sense of smell *via* implementation of highly selective and sensitive (bio) receptor elements can significantly widen the spectrum of ENs application [4], for instance in detection and analysis of the explosives. Examples of B-ENs for the detection of explosives are shown in Fig. 7.

Concept versions of the B-ENs are designed in the way allowing identification of volatile substances at very low concentrations before they reach the level hazardous to environment/people. A fundamental problem connected with proper operation of B-ENs is low activity of the biological elements in dry conditions. Detection of vapours by immobilized OBPs in the absence of an aqueous environment was clearly illustrated by Cali [125]. Among the elements of the olfactory systems, which found application in detection of the explosives, one can distinguish OSNs placed in OE responsible for detection of volatile particles and conversion of chemical energy into electric signal [126]. A method for improvement of specificity and extension of a lifetime of the OSN-based biosensors for identification of the explosives was presented by Gao et al. [26], described in the previous chapter. A novel peptide-based three-dimensional probe called “peptide matrix”, fabricated on a SPR sensor chip to enhance the sensitivity of detecting TNT was recently proposed by Komikawa et al. [115] (Fig. 7.6). As previously mentioned, the unique structure of peptide matrix was rigidly constructed by multiple TNT binding peptides fragments on SPR. A novel peptide-based three-dimensional probe called “peptide matrix” for the detection of TNT was presented by Komikawa et al. Bioinspired sensing platform, inspired by the antibody paratope region, was fabricated on a surface of SPR sensor chip to enhance the detection sensitivity (ppb/sub-ppb level) [115]. Presented peptide matrix with high sensitivity of 0.62 ppb substantially broadens the possibility of detection of small particles, including TNT. A proof-of-concept for an odour-perceptive nose-like hybrid, composed of a mechanically robust cartilage-like construct and a biocompatible biosensing platform was recently presented by Jodat et al. [123] (Fig. 7.3). The Authors speculated that hybrid constructs can lay the groundwork for functional bionic interfaces and viable humanoid cyborg nose organ [123]. In future, B-ENs can significantly overcome the imperfections of the electronic noses, especially as far as more specific and sensitive analysis of the explosives is concerned. They constitute a multidisciplinary solution to some of the bottlenecks in development of new class devices for analysis of explosive compounds. Due to high epidemiological hazard, the arrays of gas sensors should be also modified in the way enabling sampling and analysis of microbial volatile organic compounds (MVOCs) [127]. Currently, the aviation companies, such as Airbus, perform *in-situ* ENs tests to detect the explosives at the airports and in the planes, which are combined with a virus hazard identification system. It is worth to believe that when the electronics are minimized, the whole size of ENs and B-ENs can be also reduced and finally be a small facility or an accessary of smartphone. The sensing performance of Ligand Binding Proteins (LBPs) belonging to the families of OBPs and Major Urinary Proteins (MUPs) was recently evaluated by Scorscone et al. [128]. The affinity constant of 14 LBPs toward 19 chemical targets was considered in aqueous solution by competitive binding assay using fluorescence probe N-phenylnaphthalen-1-amine for insect OBPs and MUPs and 1-aminoanthracene for porcine OBPs. Presented B-EN was able to discriminate between a range of explosives and narcotic compounds with sensitivity comparable to IMS instruments (ppb range). Insights into the phage-based (B)-EN should play a significant role in the fields in need of B-ENs with precise sensing ability, including the detection of organic chemicals such as dangerous compounds, food, and disease diagnosis [124]. Optical B-EN of outstanding sensitivity and selectivity toward volatile organic compounds implemented with genetically engineered bacteriophage was recently presented by Park et al. [124]. They demonstrated rapidly responding optical B-EN with high selectivity toward gaseous explosives (Fig. 7.5). Presented concepts hold promise for the use of B-ENs in such applications. Moreover, tremendous progress in biotechnology make the future of B-ENs in explosives analysis especially promising and probable as far as their commercialization is concerned.

6. Conclusions

During last years a progress in the field of micromachining techniques, nanomaterials and biotechnology contributed to elaboration of sensitive and selective devices for detection of the explosives, which tend to achieve the operation parameters of some olfactory systems present in nature. Development of the (bio)sensors is clearly oriented towards selectivity and specificity. Implementation of new (nano/bio)materials as the active layer and better understanding of the olfaction mechanisms of vertebrates and invertebrates help to optimize the (bio)sensors and make their operation parameters close to the biological olfactory systems. Application of designed construction solutions in real systems and fulfilment of particular requirements are still a bottleneck in the process of commercialization. Enhanced sensitivity and selectivity are especially desired as far as analysis of traces of the explosives is concerned. Despite the fact that trained dogs remain a gold standard in many aspects of explosives analysis, the animals-inspired solutions open new possibilities of their replacement and complementation. The combination of synthetic biology and materials science has the potential to generate a wide range of unprecedented biomimetic materials with properties suitable to implement explosives detection systems. Fascination with the biological olfactory systems paths the road to successful implementation of such devices in real applications. High specificity and selectivity ensured by diverse elements of biological origin, with attenuated cross-reactivity for analysis of the explosives present in complex matrices allows development of reliable detection technique dedicated to explosive and hazardous materials. Additionally, increased mobility of these sensor platforms offers the possibility of implementation in field and localization of the odour sources. Identification of the explosives using the biosensors is undoubtedly one of the main options of development in the field of explosives detection and it is primarily connected with a progress in nanotechnology and biotechnology.

In conclusion, rapid and highly selective biologically-inspired devices represent a major breakthrough in the area of explosives detection. Despite the very encouraging scenario regarding the application of such devices in strategic fields in explosives sensing, limitations still persist. Improvements or milestones are required in some aspects of the biomimetic devices development:

- Optimization of biosensors' basic metrological parameters (portability, stability, lifetime, robustness, accuracy, selectivity, sensitivity, etc.);
- Translation of complex architecture of biomaterials into technological functional biomimicking materials;
- Better integration of biorecognition materials with nanomaterials and secondary transducers;
- Improving the manufacture of low-cost and highly efficient devices;
- Development of wearable devices, field-based test kits, paper strips-based bioassays, and handheld devices;
- Development of *in vivo* and *in vitro* devices;

- Improvements in explosives source localization, e.g. development of algorithms for robotic navigation;
- Stand-off/remote control systems.

To further improve the properties and functions of the artificial sensing and actuating materials, numerous efforts from multidisciplinary fields are required, particularly in-depth understanding of the principle of natural sensing and actuating systems and the biomimetic structural design for the artificial materials. Although some biomimetic devices may prove to be successful but true bio-inspiration should proceed from a more holistic view of instruments operating in their technological environment. Biologically-inspired materials chemistry seems to fit in the biomimetic trend, it shortens the distance between nature and technology. We envision that devices and machines with multiple robust functions will be formulated with the progress of advanced bio-inspired strategies, which should open avenues for many new areas.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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