ESSENTIALS OF MACHINE OLFACTION AND TASTE
ESSENTIALS OF MACHINE OLFACCTION AND TASTE

Edited by

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Preface

This book introduces the current technologies related to machine olfaction and taste, which is expected to become key technologies of human interface. The handbook of this field was published more than 10 years ago, and since then, many new technologies have been developed.

One of the new trends in the sensing field is the olfactory biosensor, utilizing olfactory receptors in a living body. Moreover, an olfactory display to present scents to a human is relatively new. In the field of taste sensors, many applications have accumulated. These important technologies have emerged during the last decade.

Thus, it is necessary to review this field thoroughly and to describe it systematically for both beginners and experts. This allows novices to learn the basics and experienced researchers and engineers to learn about the state of the art of machine olfaction and taste systematically.

This book can cover the entire range of artificial chemical sense including both olfaction and taste in a modernized way. We divide the field of machine olfaction and taste into six categories: biological matter, odor sensing technology, taste sensing technology, pattern recognition, mobile robots with olfaction, and olfactory display together with odor recorders. Thus, readers can separately learn each area from the basic to the latest research.

The primary audience of this book are people in academia and industries such as electronics, computer science, chemistry, and biology. Especially, people related to multimodal interface in virtual reality or human interface might find cues for their research or commercial products. Moreover, people in academia and industries such as communications, agriculture, medicine, advertisement, and entertainment might find this book beneficial. This book is written with the intention to be a “modern bible” and guide of machine olfaction and taste.

Takamichi Nakamoto
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Introduction to Essentials of Machine Olfaction and Tastes

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Although there are a variety of sophisticated machines for visual and auditory senses, machines for chemical senses such as olfaction and taste are still prematured. However, they are very important since they are deeply related to our primitive but fundamental capabilities. We can search for foods in daily life as well as can avoid danger using olfactory sense. Although animal’s capability is nowadays superior to human ones, we still have them. Those chemical senses cannot be ignored in our daily life. Nowadays we can create cyberspace made up of visual and auditory senses. However, that cyberspace still lacks reality since olfactory and gustatory senses are not included.

The first machine olfaction was proposed about 30 years ago. Then, it was extended and an electronic nose community appeared. Although many papers have been already published, its application toward to industry is still limited. Its sensitivity, selectivity, and robustness against disturbance should be much improved for the actual application. A variety of applications are waiting for its progress. This book describes the current effort of sensing part of machine olfaction.

Machine olfaction has another part such as olfactory display. It works as an actuator in olfaction. An olfactory display is relatively new compared with odor sensing technology. Researchers in virtual reality have focused on the olfactory display to realize cyberspace with chemical sense. Although researcher population of olfactory display is still small, it gradually spreads into the world.

A human olfactory interface has both odor sensing and olfactory display. It is now growing up in the field of human interface. Utilizing those two techniques, odor recorder and teleolfaction system are being studied.

In contrast to olfaction, a taste sensor has been applied to a certain application area. Especially, medical field is its good customer. However, we still wait for simple easy-to-use taste sensor to include taste sense in cyberspace. The attempt to realize it will be later shown.
Recently we often hear the world such as cyber-physical system. Cyber-physical system enables cyberspace with physical senses. However, we have never heard the word “cyber-chemical system.” We can have cyber-chemical system if the technologies of machine olfaction and taste are easily available.

This book describes the essential parts of machine olfaction and taste. Chapter 2 describes olfactory mechanism of a living body. Utilizing it, olfactory biosensor is being developed. Chapter 2 also explains the olfactory biosensor.

Chapter 3 shows odor sensing technology. It explains the basics of artificial sensors. Moreover, a large-scale sensor array in the same way as biological one is being studied. This trend in electronic nose is introduced in Chapter 3.

Chapter 4 shows the taste sensor. It describes the principle and its application toward foods and medicines. This chapter explains the latest research review as well as the fundamentals of taste sensor.

Chapter 5 describes the current pattern recognition technologies available in electronic noses. The pattern of many ORN responses is recognized by an olfactory neuron system. Thus, the output pattern of the array of sensors with partially overlapping specificities is recognized in machine olfaction. Chapter 5 describes the basics of pattern recognition technologies together with its advanced technologies.

Chapter 6 explains mobile robot technology with chemical senses. It can search for the target chemical in the field. Its sensor, algorithm to look for the target and the experiment is shown in this chapter.

Chapter 7 shows olfactory display and odor recorder. Various types of olfactory displays are systematically described. Moreover, the review of odor recorder is shown in this chapter.

Chapter 8 is the summary and describes the perspective of machine olfaction and taste. It describes basic part at first and then extends their contents to the advanced technology.
Physiology of Chemical Sense and its Biosensor Application

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2.1 Introduction

Odorant sensors for detecting various types of odorants are currently required increasingly for several applications, such as disease diagnosis, food administration, and risk management associated with detection of explosives and drugs. Odorant sensors based on metal-ode semiconductor devices, quartz crystal microbalances (QCM), or surface acoustic wave (SAW) detectors have been developed for a variety of odorant-detection applications. However, the performance of these sensors is still inferior to the olfactory systems of living organisms in terms of selectivity, sensitivity, and response time.

In animals olfaction plays a key role in the release of appropriate behavior under complexly changing environment. Animals extract adequate information from numerous odorants in their surroundings and respond in many aspects of the animal’s life including foraging, prey detection, finding hosts, and mating. Odor information is detected by olfactory receptor neurons (ORNs) in an olfactory organ and properly processed in neural networks in the brain and finally translated into the appropriate behavioral responses, mechanisms of which are critically important in the development of advanced odor sensors and odor tracking robots based on biological systems as well as neuroscience and neuroethology.

Transduction mechanisms in olfaction have been revealed in vertebrates and insects. Odorants are detected at the surface of the olfactory epithelium which contains ORNs in vertebrates. Insects have ORNs in the sensillum of antennae. Odorant signals detected at the
membrane of the ORNs are converted into electric signals and transmitted to the brain. In vertebrates the transduction is mediated by complex signal transduction pathways through G proteins, adenyl cyclase, cyclic adenosine monophosphate, and cyclic nucleotide-gated ion channels (i.e., G protein-coupled receptors (GPCRs)). By contrast, insect odorant receptors, coupled with an olfactory receptor coreceptor (Orco), form ligand-gated ion channels (i.e., ionotropic receptors) that control all-in-one odorant reception and ion influx. Moreover, the insect ionotropic receptors can selectively detect various types of odorants covering a wide range of chemical functional groups, including alcohols, aldehydes, ketones, acids, hydrocarbons, and aromatic compounds.

Since the transduction mechanisms in animals have been revealed, odorant receptors would be valuable odorant sensors with high selectivity, high sensitivity, and good response time compared to conventional sensors. So far a number of cell-based odorant sensors have been studied and proposed by using recent advanced gene engineering techniques. Among those cell-based sensors, insects are equipped with sophisticated molecular mechanisms that involve initial activation of odorant receptors. The insect odorant receptors would be valuable odorant sensors with high selectivity, high sensitivity, and good response time and could be assembled into a compact chip to develop portable odorant sensors.

In this chapter, first, transduction mechanisms of insect and vertebrate ORNs are introduced. In addition transduction mechanisms of insect and vertebrate gustatory signals are also introduced. Based on these findings, various kinds of biological components such as tissues, sensory neurons, proteins, and genes regarding olfaction in living organisms have been utilized for application to olfactory sensors. Different types of olfactory sensors, that is, tissue-based sensors, cell-based sensors, and receptor-based sensors, are then introduced.

2.2 Olfaction and Taste of Insects

2.2.1 Olfaction

2.2.1.1 Anatomy of Olfaction

Structure of Olfactory Sensillum
Insects detect odorants with a pair of antennae on their head and, in some dipteran species, a pair of maxillary palp extending from the base of the maxilla (Figure 2.1a). ORNs are housed in cuticular specialization, named olfactory sensillum, on these olfactory organs (Figure 2.1b, c). Olfactory sensillum has numerous minute pores (10–100 nm), named olfactory pores [57, 128], which allow odorant molecules to enter inside the sensillum. ORNs are bipolar neurons that extend their dendrites, the site of odorant reception, into the sensillum and project their axons into the antennal lobe (AL), the first olfactory center of the brain in insects [41]. Cell bodies and inner dendrites of ORNs are surrounded by three accessory cells: the tormogen, trichogen, and thecogen cells (Figure 2.1c). These cells isolate a lymph space surrounding the outer dendrites of the ORNs from the hemolymph. Differences in chemical composition of the sensillum lymph and the hemolymph generate a standing electrical potential difference, the transepithelial potential (see Refs. [58, 98]). Odorant stimulation generates a receptor potential in the outer dendritic membrane, which can induce the generation of action potentials in a more proximally located spike-generating zone. Olfactory sensilla are classified to several types according to their outer shape (s. trichodea, s. basiconica, s. coeloconica, s. placodea,
and so on). In some cases, the type of sensillum is well correlated with its function. For example, s. trichodea in male moths house ORNs specifically tuned to conspecific sex pheromones [52, 53], while others house ORNs for so-called general odorants such as from foods or plants [108].

**Detection of Odorants by ORNs**

Odorant molecules in the air are first absorbed on the cuticular surface of the sensillum, and then they diffuse inside the sensillum through olfactory pores and pore tubules [52, 54, 55]. Since most volatile odorants are hydrophobic in nature, it is difficult to efficiently pass
sensillum lymph to dendritic membrane of ORNs. Therefore, mechanism to facilitate solubilization of odorants into aqueous lymph layer is important to achieve sensitive detection of odorants. For this, insects utilize small (about 15 kDa) soluble globular proteins named odorant binding protein (OBPs) [141] that bind odorants and transport them to dendritic membrane of ORNs. Mechanisms of odorant binding and release by OBPs are well studied using the silkmoth (Bombyx mori) pheromone-binding protein 1 (BmPBP1) that binds sex pheromone components of that species. BmPBP1 has two different conformations that reversibly change in a pH-dependent manner [149]. At neutral pH, odorant binding pocket located inside of proteins is open for binding odorant, while at acidic pH C-terminal loop domain of PBP occupies this binding pocket [42, 73]. This conformational transition is believed to occur around dendritic membrane due to lower pH around cellular membrane, resulting in the release of odorant from internal binding pocket around ORs. Then, odorants are detected by OR complex that activate chemoelectrical transduction machinery on dendritic membrane of ORNs.

2.2.1.2 Signal Transduction of Odor Signals

Upon binding to OR, the information of odorants is converted into electrical signals in ORNs. Earlier studies have reported rapid and transient increase of G protein-mediated second messenger, inositol triphosphate (IP3), in the antennal homogenates after pheromone stimulation [11]. Expression of heterotrimeric G protein in ORNs and activity of its effector enzyme in antennal homogenate were also demonstrated, suggesting that odorant signals are transduced into electrical signals via heterotrimeric G protein-mediated second messenger cascade (Figure 2.2a) [48, 67]. However, recent physiological analysis of ORs revealed that insect ORs form heteromeric complex with their coreceptor Orco (originally named as Or83b in Drosophila melanogaster) and function as an odorant-gated ion channel (Figure 2.2b, c) [121, 126, 147]. Orco is originally isolated as a member of insect ORs and has the following unique characteristics [68, 100, 144]: (i) Orco is exceptionally conserved across insect species, while conventional ORs are highly divergent within and across species. (ii) Orco is expressed in most ORNs, while conventional ORs are expressed in specific subsets of ORNs.

Sato et al. coexpressed BmOR1 with BmOrco and other combinations of members of the Orco family with ORs in heterologous expression systems. Examination of the electrophysiological properties of an Orco/OR complex revealed that it acts as a pheromone/odorant-gated nonselective cation channel (Figure 2.2b) [121]. Interestingly, there was no evidence for an elevation of second messenger levels upon stimulation with ligands, indicating no involvement of a G protein-mediated cascade in the activation of Orco/OR complexes. Later pharmacological analysis of cultured cells coexpressing Orco and ORs from D. melanogaster supported this conclusion [126]. In the meanwhile, Wicher et al. found that fast transient and slow prolonged ion currents occur in cultured cells coexpressing DmOrco and D. melanogaster ORs upon stimulation with appropriate ligands for the expressed ORs (Figure 2.2c) [147]. They proposed that fast currents result from direct activation of Orco by ORs and slow currents occur via G protein-mediated activation of Orco. Both studies indicated that odorant signals are mediated by odorant-induced channel activity of ORs/Orco complex or Orco but different in terms of involvement of G protein-coupled pathway. Further studies will be required to reach consensus about the roles of G protein-mediated second messenger system on reception of odorants.
In this regard, phosphorylation of ORs by protein kinase C activated by presumably G protein-mediated second messengers reportedly enhances responses to odorants [36, 148], suggesting that second messenger system may not directly activate but modulate activity of Or/Orco channel through phosphorylation of ORs. More recently, it was reported that latency of electrophysiological responses of antennae of several insect species is as fast as several millisecond order [132]. This response speed is in accordance with the range of ionotropic pathway, indicating at least fast response is mediated by ionotropic activity of OR and Orco complex.

2.2.1.3 Molecular Biology of Olfaction

The insect OR gene family was first identified from the fruit fly by bioinformatics-based methods as well as large-scale screenings of olfactory tissue-specific genes [25, 34, 143]. Sixty OR genes are found in whole genome sequence of the fruit fly [144]. After that OR genes have been identified from various insect species. The number of OR genes considerably
varied between species ranging from 10 in the body louse to more than 300 in ants. Amino acid sequence comparison revealed that insect ORs form a unique gene family with no obvious homology with any other proteins including ORs from vertebrates. Although insect ORs possess seven-transmembrane domain characteristic to GPCR family, they have a reverse membrane topology compared to GPCRs with their N-terminal on the cytoplasmic side and C-terminal on the extracellular side [7, 49, 72, 89]. Indeed, recent physiological studies demonstrated that insect ORs form odorant-gated ion channel with Orco (see Section 2.2.1.2 in detail).

Response Profiles of ORs
In an OR and Orco channel complex, OR is responsible for ligand binding and determines response profiles of ORNs [121]. By now, response spectrum of more than 100 ORs has been determined by using “empty neuron” expression system in the fly antennae and/or heterologous cell expression systems such as *Xenopus* oocytes. In principle, each OR can bind different odorants and each odorant can be recognized by multiple ORs. Response spectrum of individual OR continuously distributed from narrowly to broadly tuned one [17, 38, 145]. Comprehensive analysis using the fruit fly has uncovered the relationship between ORs and ORNs as well as ORNs and glomerulus in the AL [28, 33]. Similar to vertebrate olfactory systems, most ORNs selectively express one of many ORs, and ORNs expressing the same OR project into a single defined glomerulus in the AL. Since each OR normally responds to various odorants and each odorant is detected by various ORs, odorant information is represented as a combination of activated glomeruli in the AL.

2.2.2 Taste
In the gustatory system, the sense of taste is essential for the animals to evaluate which food is good to eat and which food should be avoided. Compared to most mammals that can discriminate five basic tastes (see Section 2.3.2.1), insect basic tastes are divided into four categories: bitter, sweet (sugar), salty, and water. In addition to these tastants, insect gustatory system can detect uncanonical taste substances such as fatty acids, sour tastes, and chemicals unrelated to food such as contact pheromones. In this section, the mechanisms of taste detection in insects are briefly described.

2.2.2.1 Anatomy of Taste

Taste Organ
One of the striking features of the insect gustatory system is that taste organs are not restricted to mouth part but are distributed in multiple body parts. For example, in adult fruit flies, four appendages—the proboscis, legs, anterior wing margins, and ovipositor—possess gustatory function (Figure 2.3a) [79, 129, 142]. The proboscis is a long appendage extending from the head and comprises external taste organ named labella that is located at the apical end of the proboscis and three internal organs—the labral sense organs (LSOs), the dorsal cibarial sense organ (DCSO), and the ventral cibarial sense organ (VCSO) that are located along the pharynx (Figure 2.3b) [129]. These organs play roles in determining whether to ingest or expel food and thus can be regarded as the functional equivalent to the mammalian tongue. Taste organs
are present on distal segment of legs, tarsi. Taste sensors on tarsi carry out initial sampling of potential food and evaluate the quality of it. Tarsal taste organ on male forelegs also plays a role in detecting contact pheromones that promote or inhibit courtship behavior of males [10]. Taste organs on wings are indicated to participate in the detection of microbe-derived lipopolysaccharides that induce grooming behavior to remove microbe from fly’s cuticle [157]. The ovipositor taste organ provides information of nutrient conditions to identify location suitable for egg laying [159].

**Structure of Taste Sensillum and Gustatory Receptor Neurons**

Taste substances (tastants) are detected by sensory neurons referred to as gustatory receptor neurons (GRNs) housed in taste sensillum on those organs. GRNs are bipolar neurons that extend their dendrite into the shaft of taste sensillum and project their axon to the suboesophageal ganglion, taste center in insect brain. In contrast to olfactory sensillum that has many pores on its cuticle, taste sensillum has a single pore at the apical end of the sensillum from which tastants enter into taste sensillum. Typically, there are one to four GRNs and one mechanosensory neuron in individual sensillum. Each GRN is tuned to substances of one of four basic taste categories.

**2.2.2.2 Molecular Biology and Signal Transduction of Taste**

Recent studies have revealed that tastants are detected by various types of receptors expressed in GRNs. Basically, the types of receptor correspond to taste categories. In this section, the types of receptors and signal transduction activated by interaction of tastants with receptors are summarized.

**Bitter and Sweet (Sugar) Taste**

Bitter and sweet tastes are detected by the large receptor family named gustatory receptor (GR) that is the major class of insect taste receptor. GR was first discovered from the fruit...
fly by bioinformatics approach of nearly completed *Drosophila* genome sequences to seek candidate genes that can encode seven-transmembrane domain receptor [26, 123]. By these analyses, 43 GRs that belong to the novel membrane protein family and expressed selectively in subsets of GRNs were reported. Later analysis revealed that there are 68 GRs in whole genome sequences of *Drosophila* [114]. By now, GR family genes are reported from various insect species of different orders such as mosquito, moth, beetle, wasp, bee, aphid, and louse. Number of GR genes is different between species from 10 in honeybee *Apis melifera* to 114 in disease vector mosquito *Aedes aegypti*. Similar to insect ORs, amino acid sequences of GRs are highly divergent within and among insect species. Although GRs have seven-transmembrane domain, they do not share homology with other known GPCRs and membrane receptors and form independent receptor family. In this sense, membrane topology analysis of a GR from the silkmoth indicated that, like insect ORs, GR has inverted membrane topology compared to canonical GPCRs [164]. The closest relative of GR family is the insect OR family. Phylogenetic analysis revealed that the emergence of the GR family preceeds that of the OR family, suggesting that ORs may evolve from GRs [114, 140].

GRs basically function as bitter and sweet taste receptors. Members of the GR family function as receptors for other categories such as an amino acid (l-canavanine) [29], nonvolatile contact pheromones [10], and CO$_2$ [130]. Surprisingly, it is reported that member of GR family is also involved in the detection of nonchemical signals including light [155] and temperature [91], indicating highly divergent roles of GRs.

In many cases multiple GRs are expressed in individual GRNs, suggesting that GRs form heteromeric complex to exert their functions. In the meanwhile, at least 2 GRs tuned to fructose can be functionally reconstructed in heterologous expression system [122]. Thus, mode of action of GRs is still largely unknown.

Signal transduction pathway following activation of GRs is also a major open question in the insect gustatory system. Recent studies raise the possibility that GR signaling is mediated by both G protein-coupled metabotropic pathway and ionotropic activity of GRs. Regarding metabotropic pathway, the expression of heteromeric G protein in GRNs has been shown [134], and mutation or knockdown of effector gene of G proteins reduced physiological and/or behavioral responses to bitter and sweet tastants [29, 50, 60, 136]. The involvement of G protein-coupled pathway is also evident for tastants in other categories but mediated by GRs, including CO$_2$ [160] and an amino acid (l-canavanine) [29]. Regarding ionotropic activity of GRs, at least one GR for fructose appears to function as a tantant-gated nonselective cation channel, independent of a G protein-coupled pathway [122].

**Salty Taste**

Insects equip two types of salt GRNs: one tuned to high salt and the other to low salt. In *Drosophila* larvae, two epithelial Na channel (ENaC) family members, PPK11 and PPK19, are required for response to low salt [81], whereas in adult flies member of IR family IR76b is required for low-salt detection [165], which encodes continuously open-state Na$^+$ leak channel. Because Na$^+$ concentration in sensillum lymph is much lower than that of the hemo-lymph, influx of Na$^+$ through ENaC and IR76b occurs when insects take food containing low salt. This influx depolarizes the GRNs. Identification of receptors responsible for detection of high salt is a major question of salt detection in insects.