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Advances of Molecular Imaging for Monitoring the Anatomical and Functional Architecture of the Olfactory System

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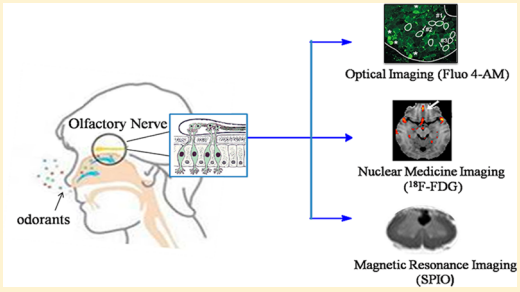
ABSTRACT: The olfactory system of organisms serves as a genetically and anatomically model for studying how sensory input can be translated into behavior output. Some neurologic diseases are considered to be related to olfactory disturbance, especially Alzheimer's disease, Parkinson's disease, multiple sclerosis, and so forth. However, it is still unclear how the olfactory system affects disease generation processes and olfaction delivery processes. Molecular imaging, a modern multidisciplinary technology, can provide valid tools for the early detection and characterization of diseases, evaluation of treatment, and study of biological processes in living subjects, since molecular imaging applies specific molecular probes as a novel approach to produce special data to study biological processes in cellular and subcellular levels. Recently, molecular imaging plays a key role in studying the activation of olfactory system, thus it could help to prevent or delay some diseases. Herein, we present a comprehensive review on the research progress of the imaging probes for visualizing olfactory system, which is classified on different imaging modalities, including PET, MRI, and optical imaging. Additionally, the probes' design, sensing mechanism, and biological application are discussed. Finally, we provide an outlook for future studies in this field.



KEYWORDS: *Molecular imaging, olfactory system, molecular probe, olfactory bulb, PET*

1. INTRODUCTION

The olfactory system is a special brain structure involved in odor sensation that has important implication to basic neuroscience research, like dendrites transmitting and early disease diagnosis.[1] It is an interesting and accessible system for delivering olfactory activity that comprises three important components, including olfactory neuroepithelium, olfactory bulb (OB), and olfactory cortex. OB is an important part of the olfactory system which has diverse neuronal organization and anatomical structures to receive and transduce odorant information to the olfactory system. And how is an odor map transferred from afferent terminals to postsynaptic dendrites? It is essential to realize this complex process relies on the axonal connection and projection. First, the odorant molecules are detected by the olfactory receptors (ORs) which are the initial players in a signal transduction cascade.[2] Each OR binds to several odorants, and each odorant is detected by a specific combination of ORs. The mouse, for example, has approximately 1200 ORs, while humans have less than 400. These receptors are members of the class A rhodopsin-like family belonging to G protein-coupled receptors (GPCRs).[3] ORs are expressed in the cell membranes of olfactory receptor neurons (ORNs). Every ORN expresses only one OR. In vertebrates, ORs are located in both the cilia and synapses of the olfactory sensory neurons (OSNs), which occupy a small area in the upper part of the olfactory epithelium (OE).[4] And ORs are also located on the antennae and other chemosensory organs in insects.[5] OSNs converge onto either one or a few specific glomeruli in the OB, and individual odorants elicit specific spatial patterns of glomerular activity.[6] In response to the odorants, the activated ORs convert the chemical odor stimuli into a nerve impulse resulting in an elevation of cAMP levels.[7] Then, odor information is transferred to the various principal neurons that form the glomerular module. Eventually, the stimulus is transmitted to the brain where the odor is ultimately perceived.

To be specific, the olfaction is generated by transmitting odor molecules to the OB and passing through several anatomic layers, as shown in Figure 1. Then odorant signals are transferred to the glomerular layer (GL) by way of olfactory nerve layer (ONL) which is the most superficial layer of OB formed by the ORN axon.[8] The mitral cells as the majority of projection neurons of the mitral cell layer (MCL) form the MCL. The layer that was intermediate to the GL and MCL was defined as the external plexiform layer (EPL).[9] Granular cells (GC) play critical factors in the glomerular module and form synapses on the soma and dendrites of mitral and tufted cells. The area of EPL even has the extension and branches of GC.

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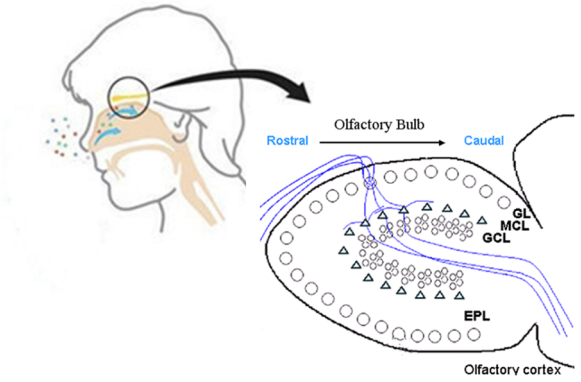

Figure 1. Scheme for neural pathway and construction inside olfactory bulb which plays a key part in transmitting odors from nasal cavity, shown in the sagittal orientation. GL, glomerular layer; MCL, mitral cell layer; GCL, granular cell layer; EPL, external plexiform layer.

These neurons project their axons to glomeruli, which are specialized structures of neuropil in the olfactory bulb.[10] In general, the ORs have a key role within the olfactory system in recognizing and discriminating numbers of structurally diverse odorous molecules.[11] Further evidence confirmed that several neurologic diseases are associated with olfactory disturbance, such as Parkinson's disease (PD), multiple sclerosis, Alzheimer's disease (AD), and so on. As a useful biomarker for early Parkinson's, olfactory dysfunction is common in PD and often predates the clinical diagnosis.[12] Some come forth that in the early phase of PD, the changes of diffusion imaging arguments are visualized in the olfactory tract and substantia nigra. Therefore, we can ensure that monitoring olfactory dysfunction can provide a potential method to indicate the process of PD. However, it is still not well understood how the function and mechanism of olfaction in olfactory system are manifested at the cerebral level, although recent developments in techniques have made great efforts to examine this question in greater depth.

Molecular imaging can be defined as the visual representation, characterization, and quantification of biological processes at the molecular and cellular levels in living organisms. It is a rapidly developing biomedical research discipline that extends these findings in living organisms to a more meaningful dimension. And it provides varied applications, including early detection and characterization of diseases and evaluation of treatment in living subjects.[13] Furthermore, molecular imaging is similar to a biopsy but is done noninvasively, in real time, and with potential for longitudinal monitoring. Applying molecular imaging probes, we can visualize the structure of olfactory system and map the olfactory code accurately as many interesting work developed. In this Review, we explored the anatomical and functional architecture of olfactory system using several imaging probes which is according to the different imaging techniques, including positron emission tomography (PET), magnetic resonance imaging (MRI), and optical imaging.

2. IMAGING OLFACTORY SYSTEM WITH FLUORESCENT PROBES

Fluorescent imaging is widely used in olfactory research field due to its great temporal and spatial sampling capability as well as high selectivity and sensitivity both in vivo and ex vivo. So it has become one of the most important sensing technologies for the olfactory system, since it is applying fluorescent probes which realize the measurement of olfactory activity. In this part, the fluorescent probes are classified based on their chemical structures.

2.1. Protein Based Fluorescent Probes. With appropriate techniques, Ca^{2+} signals can be recorded at many levels of complexity, ranging from large scale neuronal networks down to individual presynaptic boutons or postsynaptic spines. It has been applied to track neuronal activity especially active ORNs and discriminate it from neighboring similar active ORNs. Measured with electrophysiology, the kinetics of Ca^{2+} response is related to the odor-evoked activity of the ORNs and shown in an ORN that can be identified as an odor-evoked cell by Ca^{2+} imaging. Therefore, imaging Ca^{2+} has become an important approach to analyze the encoding and processing of olfactory information by populations of glomeruli or neurons. Genetically encoded probes have obvious advantages including noninvasive loading procedure, application for long-term studies and the ability to target selected neurons. Here we first discuss the adaptability of genetically encoded Ca^{2+} indicators for in vivo Ca^{2+} imaging (Table 1).

As the valuable tools to detect Ca^{2+} kinetics, genetically encoded fluorescent calcium indicator proteins (FCIPs) can be applied in both in vitro and in vivo. Camgaroo-1 and camgaroo-2 (Cg1 and Cg2) are two variants of yellow emitting fluorescent proteins (YFP) constructed by inserting calmodulin (CaM)[14] which is expressed to image calcium dynamics in Drosophila mushroom bodies that intensively investigated for its role in olfactory learning and memory (Figure 2a, c).[15] Cg2 can be applied on the transgenic mouse line MTH-Cg2−19. Under the control of the tetracycline-inducible promoter, Cg2 was showed to respond odor stimulation in the OB especially in afferent sensory axons and granule cells.[16] G-CaMP, a single-

Table 1. Genetically Encoded Probes Expressed in the Olfactory System

probe	GFP variant	site of expression	organism model	ref
synapto-pHluorin	pH-sensitive mutant of GFP	olfactory receptor neurons, the projection neurons, and the interneurons	Drosophila	24
		olfactory sensory neurons	OMP-spH mice	26
camgaroo-1	YFP	mushroom bodies	Drosophila	8
camgaroo-2	YFP	mushroom bodies	Drosophila	
		olfactory afferent sensory axons and granule cells	MTH-Cg2-19 mice	10
G-CaMP	cpGFP	olfactory sensory neurons and projection neurons	Drosophila	12
G-CaMP 2		dorsal of olfactory bulb	OMP-tTA/tetO-G-CaMP2 mice	15
G-CaMP 3		mushroom bodies	Drosophila	16
		mitral cells, tufted cells and glomerular interneurons	Thy1-GCaMP3 mice	17

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Table 2. Spectrum Information of Optical Dyes

no.	optical probes	λ_{ex} (nm)	λ_{em} (nm)
1	Calcium Green-1	506	531
2	Oregon Green 488 BAPTA-1	501	526
3	Rhod-2-AM	549	581
4	Fluo-4-AM	491	516
5	Alexa 488	490	525
6	Alexa 594	590	617
7	Di-8-ANEPPQ	514	714
8	RH-795	489	685
9	RH-1838	630 ± 10	>670
10	Cu_2(FL2E)	480	522

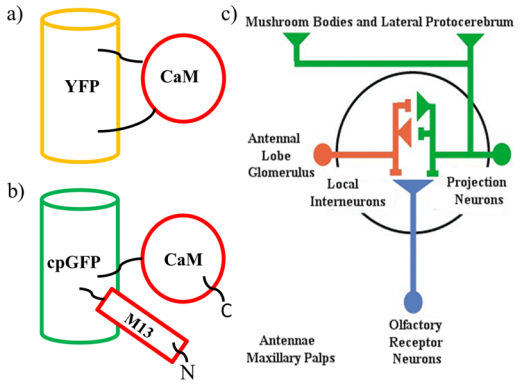

Figure 2. Yellow (YFP) and green (GFP) variants of *Aequorea victoria* fluorescent proteins as two genetically encoded fluorescent protein Ca^{2+} sensors: (a) Camgaroo and (b) G-CaMP. Calmodulin (CaM), the calmodulin-binding polypeptide M13 (M13), is inserted into YFP (a) or attached to a cpGFP (b). (c) A glomeruli activity's circuit model in the Drosophila antennal lobe which contains mushroom bodies. The cell bodies of olfactory receptor neurons (ORN, shown as blue line) belong to the antennae and maxillary palps. In the specific glomeruli of the antennal lobe, they organize excitatory synapses with projection neurons (PN, shown as green line) and local interneurons (LN, shown as red line). PNs conduct the olfactory information to mushroom bodies and lateral protocerebrum and are considered to make collective synapses with LN.

wavelength intensity modulating Ca^{2+}-sensitive probe, is reconnected by two fused circularly permuted green fluorescent proteins (cpGFPs) (Figure 2b).[17] It has been successfully used to image populations of glomerular activity in the Drosophila antennal lobe presynaptically or postsynaptically expressed in ORNs or in projection neurons.[18] Odors eliciting specific patterns of glomerular activity were conserved in different flies, and the specific responsivity of a given glomerulus was considered to be the consequence of a single OR expressed by the incoming ORNs. Then, G-CaMP has been extended to analyze the mushroom bodies of Drosophila which observed spatially special odor-evoked activity.[19] Besides, the G-CaMP under a potassium-channel promoter especially expressed in mitral cells, tufted cells and a part of juxtaglomerular cells using a transgenic mouse.[20] Additionally, the study evaluated the spatial presentation of chemical features with surveys of odor-evoked responses expressing G-CaMP2 in the dorsal OB of heterozygotic OMP-tTA/tetO-G-CaMP2 mice (OMP, olfactory marker protein).[21] With this method, relatively low odor concentrations in sparsely activated regions could be well examined for the first time. For example, a two-photon Ca^{2+} imaging study indicated that G-CaMP3 was expressed in the Drosophila MB to monitor the activity of MB neurons in vivo.[22] And G-CaMP3 could be expressed in the mouse OB to functional map three neuronal populations (mitral cells, tufted cells, and glomerular interneurons).[23] Currently, fiber-optic microscopy allows the in vivo imaging of a fluorescently labeled neural system in mice. It is the only approach for noninvasive access to the olfactory epithelium and nerve until now.[24] Thus, the fiber-optic probe could be widely applied to image cell bodies of ORNs and bundles of the olfactory nerve by the model of CaMK−eGFP transgenic mice.[25] An estimable work indicated that genetically encoded Ca^{2+} indicators have been used extensively in invertebrates and are expected to become more important for those studies in vertebrates.[26] Further, they applied antiserum to visualize drosophila tachykinin (DTK) peptide distribution in relation to Gal4-driven GFP and observed presynaptic Ca^{2+} and synaptic transmission in the ORNs. This finding indicated that the ORNs were restrained by DTK applied the antennal lobe.[27] TLR2-LUC-GFP transgenic mice were provided under transcriptional control to image microglial activation and Toll-like receptor 2 (TLR2) responses in vivo from ischemic brains. It is reported to be a good model with the two-tier reporter system luciferase (LUC) and GFP by biophotonic and bioluminescence molecular imaging.[28] In the brain immune response, observing the TLR2 response of OB microglia proposed that OB microglial cells could serve as sensors of brain inflammation. After labeling cells in the subventricular zone (SVZ) by stereotaxic injection of a GFP-expressing lentivirus or Cell Tracker Green, the Cell-vizio system has been proved to visualize cells in real time to follow adult-born neuroblasts migrating from the SVZ to the OB in living mice which applies an integrated fibered confocal fluorescence microscope.[29] Another interesting study completed by Tsai and Barnea has important implications for how newly regenerated OSNs find the correct glomerulus. And using gal and GFP generated from designed alleles can stain for transgenic or endogenous MOR28 OSNs, respectively.[30] Consequently, this study indicated the fact that OSNs with a specific odorant receptor coalesce on a single (or very few) glomeruli.

Synapto-pHluorin (spH) is targeted expression for OSNs, since it contains a pH-sensitive GFP whose fluorescence is quenched at acidic conditions. This indicator was demonstrated to be capable of reporting responses reliably, with appropriate signal amplitudes and signal-to-noise ratios. In Drosophila, two studies monitored the synaptic activity with odor evoked by spH. One showed that the activity can be visualized in three populations of neurons in the antennal lobes, ORNs, the projection neurons, and the interneurons.[31] The other elucidated that a formation of memory trace by synaptic recruitment is yielded by an olfactory classical conditioning under the study of antennal lobes.[32] In 2004, an interesting experiment applied with genetically encoded probes in an OMP-spH mice model, and found that the spH expressed in mature OSNs.[33] In this model, green fluorescence was observed to be localized in the glomeruli of the OB, and low-intensity fluorescence was labeled in the superficial nerve layer containing the axons of OSNs. Later, to measure presynaptic inhibition, it was the first time to characterize three pathways (intrinsic paired-pulse depression of release, intraglomerular, feedback inhibition of release, and lateral, interglomerular inhibition).[34] Their data disclosed that the dimensions of OSN input to the glomerulus were modulated by intraglomerular feedback inhibition but that inhibition played little or no part in modeling the glomerular input.

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Originating in the brainstem raphe nuclei prior, serotonergic fibers preferentially projected into the glomerular layer, where ORN express the same receptor gather to form glomeruli onto mitral cells. To examine the serotonergic modulation of sensory input to olfactory glomeruli, Petzold and co-workers imaged the synaptic activity of ORN in glomeruli using OMP-spH mice that express spH in ORNs.[35] Their results pointed out that glomerular activation after odor stimulation was attenuated by increased serotonin 2C (5-HT2C) receptors activation and magnified by 5-HT2C receptor inhibition, resulting in the increased activation of GABAB receptors on ORN. The discovery revealed a noted modulation of sensory input by the serotonergic system at the first stage of synaptic processing of odor information. In addition, it proved that visualizing individual glomeruli which performed in OMP-IRES-spH mice by using electroporation of tetramethylrhodamine-dextran under the guidance of a two-photon microscope.[36]

2.2. Antibody Based Fluorescent Probes. To date, there is only few works done in this field. Anti-HuC/D antibody immunopositivity was discovered in cells migrating from the olfactory neuroepithelium toward the telencephalon in the chick embryo by means of immunofluorescence and confocal laser microscopy. It is expressed that the anti-HuC/D can label the olfactory migratory cells which are the only ones that expressed the HuC/D antigens.[37] To confirm this, Treloar et al. made the tracks that axons from OSNs expressing a specific odorant receptor project to populations of glomeruli in the OB and ONL by the adult M72-IRES-tauGFP mice.[38] Using analysis of the GFP immunolocalization within M72 glomeruli, it was found that the majority of the OSN axons innervating glomerulus express the same odorant receptor.

2.3. Small Molecule Based Fluorescent Probes. Small molecule based fluorescent probes for imaging olfactory system contain varied chemical structures with different spectrum information (Table 2). As shown in Figure 5, the calcium-sensitive dye Calcium Green (**1**) dextran was loaded into silkmoth brain neurons by iontophoretic application.[39] The normal approach was inserting a micropipet coated with the dye into macroglomerular complex or the ordinary glomerular region. However, this method was used only for antennal lobe projection neurons. Another probe, Ca^{2+} indicator dextran-conjugated Oregon Green BAPTA-1 (**2**), was guided by two-photon imaging of OMP-spH mice which express the spH protein in a target glomeruli.[40] Using an electroporation method, it was able to clearly visualize multiple neurons that were all accompanied by a single target glomerulus in GL, external plexiform layer, and even mitral cell layer. Although only a small population of neurons within a glomerular module was labeled in each trial, these data enabled one to visualize the anatomical connectivity within a glomerular module and compare odorant response properties between multiple neuronal subtypes associated with the same glomerulus.

Besides, the Ca^{2+} indicator rhod-2-acetoxymethyl ester (rhod-2-AM, **3**) was used to image the zebrafish OB. It was found that odor stimulation evoked Ca^{2+} signals in OB were strong, stable over hours and odor-dependent. By the application of rhod-2-AM, cytoplasmic fluorescence could remain high for more than 8 h.[41] While, another calcium-sensitive dye, fluo-4-AM (**4**), was used for imaging Ca^{2+} on slices of the *Xenopus laevis* tadpole olfactory mucosa. With the probe **4**, the increase of the intracellular calcium concentration indicated that some ORNs responded to stimulation with amino acids. And it displayed that ORNs of this animal model have two olfactory transduction pathways: cAMP-dependent and cAMP-independent.[42] In parallel, based on fluo-4-AM, the ORNs of lobster, as a large olfactory organ which is a beneficial invertebrate animal model for easy access to the OSNs, were elucidated to generate a variation of intracellular calcium in response to odorant activation that could monitor all ORNs activities and roughly visualize three clusters of ORNs.[43] Dextran-conjugated Alexa 488 (green) and Alexa 594 (red) (**5, 6**) were also used as the tracer injected in OB of OMP-spH knock-in mice with a two-photon microscope and dyes applied to visualize the target glomerulus.[44] The results showed that the most red-labeled neurons were tufted cells, and the majority of the green-labeled cells were mitral cells. Although mitral and tufted cells received similar odor signals from a shared glomerulus, this experiment indicated that the odor information was processed in different ways and their output was sent to different higher brain centers via the piriform cortex (PC) and olfactory tubercle.

In addition, the voltage-sensitive dyes have been extensively reported to image the olfactory information, especially in the serials of RH. Generally, as binding to the plasma membrane in ways, these dyes are thought to function as voltage transducers that allow their fluorescence or absorption to be modulated by changes in transmembrane voltage.[45] To examine how odorants are represented at the olfactory processing, a voltage-sensitive dye Di8-ANEPPQ (**7**) was labeled anterogradely at afferent axons of ORNs in zebrafish.[46] The activity was recorded optically by different odorants in afferent axons and across the array of glomerulus with the dye. The consequences confirmed that certain subpopulations of the OB were preferentially activated by defined natural odorants. The olfactory input is relayed to the hippocampus via synapses interactions among the PC and entorhinal cortices (EC). Accordingly, to further check on the absence of EC to PC interactions after lateral olfactory tract stimulation, high-quality molecular imaging of neuronal activity was performed with the voltage-sensitive dye RH-795 (**8**) in the isolated guinea pig brain.[47] Eventually, the optical imaging indicated that odor-evoked activity in the EC did not induce massive PC activation. Probe RH-1838 (**9**) was infused in the rat main olfactory bulb (MOB) to explore the spatiotemporal dynamics of odor evoked activity.[48] These results demonstrated that in the MOB the represented odor identity and concentration are represented by spatiotemporal patterns, rather than spatial patterns alone. The present data confirmed that OB is abundant in NO-producing cells and is an ideal spot to clarify the functions to NO.[49] A trappable probe Cu_2(FL2E) (**10**) was reported to respond rapidly and selectively to endogenously produced NO in OB cell cultures.[50] Thus, resting levels of NO and odor-evoked changes in NO can be directly measured in the OB. Sialylated glycans could be labeled in living cells by providing the sialic acid biosynthetic pathway with *N*-acetylmannosamine (ManNAc). According to this fact, a study labeled the zebrafish embryos with Alexa-488 or 555 conjugate of difluorinated cyclooctyne (DIFO) after incubating in peracetylated ManNAc (Ac4ManNAz), which provides metabolic labeling of cell-surface sialylated glycans (Figure 3).[51] In this olfactory organ, DIFO-488-labeled glycans were visualized in both apical and basal parts of the epithelium at the olfactory pit, while DIFO-555-labeled glycans were expressed in the apical regions and periphery of the epithelium. Another group used peracetylated *N*-azidoacetylgalactosamine (Ac4GalNAz) to metabolically label zebrafish with DIFO-Alexa-647 conjugate as the image probe[52] By analyzing the

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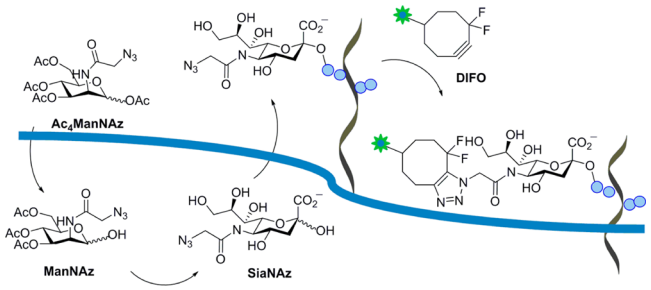

Figure 3. Scheme of metabolic visualizing sialylated glycans. Cell-permeable Ac4ManNAz enters cells and cleaves its acetyl groups to obtain ManNAz which participates in the process of the sialic acid biosynthetic pathway. Then Ac4ManNAz converts to SiaNAz, which is conjugated with cell-surface glycans by sialyltransferase enzymes and lightened by the Alexa Fluor-conjugated DIFO reagents.

uptakes in the olfactory organ, it was found that the more recently produced glycans were located only in the olfactory pit, whereas older glycans were observed in both the olfactory pit and epithelium.

Inhalation is an important route of exposure. It is reported that nanoparticles not only can reach the circulation from lungs, but can also potentially reach brains via the olfactory nerves. To evaluate whether quantum dots (QDs, CdSe/ZnS nanocrystals) could be transported from the olfactory tract to the brain, adult C57BL/6 mice were exposed to an aerosol of QDs for 1 h via nasal inhalation.[53] As shown in Figure 4a, this transport was

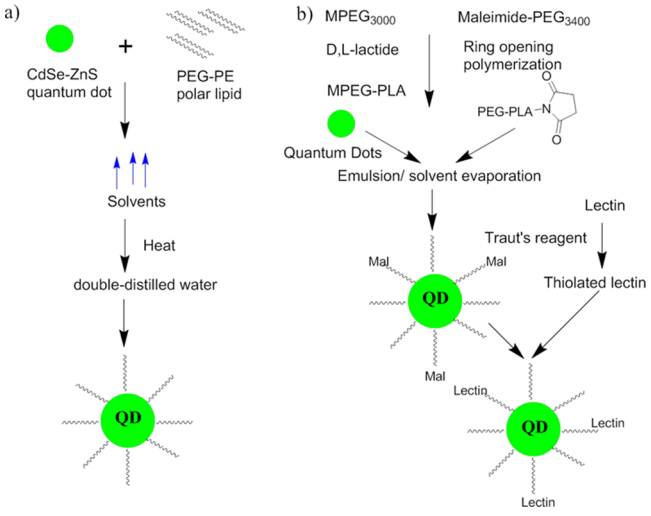

Figure 4. (a) Scheme of QD encapsulation that numerous PEG−PE molecules form a self-assembled micelle around a QD. (b) Preparation of WGA-QDs-NP. The nanoparticles of QDs-loaded PEG−PLA were synthesized by a blend of maleimide-PEG_{3400}-PLA_{54000} and methyl-PEG_{3000}-PLA_{49000} with evaporating solvent. Then, adding thiolated lectin to maleimide 1:3 into the QDs-loaded nanoparticles and incubating with 2-iminothiolane-thiolated WGA could ultimately get WGA-QDs-NP.

confirmed by visualizing QDs with a variety of microscopy techniques, ranging from fluorescent to transmission electron microscopy. It has been well-presented that wheat germ agglutinin (WGA) horseradish peroxidase focused on the OB after intranasal administration to rats. A similar excellent work was performed among which the WGA-QDs-NP was synthesized by the combination reaction of tri-*n*-octylphosphine oxide coated CdSe/ZnS QDs (TOPO-QDs) with PEG−PLA shells, and the conjugation of the obtained nanoparticles with WGA at the terminals of PEG spacers (Figure 4b).[54]

3. IMAGING OLFACTORY SYSTEM WITH NUCLEAR MEDICINE (PET/SPECT) PROBES

X-ray computed tomography (X-CT) was used to investigate olfactory disorders formerly, which has proved to be useful but with very limited application. Meanwhile, advanced functional neuroimaging techniques, such as PET, SPECT and functional magnetic resonance imaging (fMRI), have been employed in the brain's olfactory processing. As one very sensitive and accurate method, PET could directly reflect the change of regional cerebral blood flow (rCBF) which is tightly regulated to meet the brain's metabolic demands. Therefore, PET could assess central neural circuits involved in the olfactory processing. PET usually employs a trace amounts of radioactive probe containing a positron emission isotope such as carbon-11 (physical half-life 20 min), nitrogen-13 (physical half-life 10 min), oxygen-15 (physical half-life 2 min), and fluorine-18 (physical half-life 109 min). Among several PET radionuclides used for incorporation into biomolecules, the radioactive tracer $H_2{}^{15}O$ which can reflect rCBF is generally used. This is due to the tracer's short half-life that accepts the planning of several experimental conditions in a single session. The first noteworthy study in healthy humans used the ^{15}O-labeled probes PET to measure rCBF.[55] The study clarified that the two mainly foci were lied in the conjugation of the temporal and inferior frontal lobes in two hemispheres. The subsequent studies first reported the exception to the strong asymmetry in right and left orbitofrontal cortex (OFC). Measuring rCBF with PET when exposing healthy humans to highly disgusting olfactory stimuli (such as dimethyl sulfide, ethanethiol, methanethiol), the strong rCBF rise were observed in the left OFC.[56] By PET scans, a later work suggested the functional relative between the amygdala and OFC was in both hemispheres. It revealed a strong connection between increases of rCBF in the left amygdala and OFC in response to disgusting odorants when attempting only detection of an odor. Dade and colleagues explored the brain function using PET in various stages of olfactory memory processing and discovered a weak ambilateral activity in the short term recognition state and a strong double side activity in the long term recognition state.[57] These findings for the first time uncovered the dynamic nature of links between the amygdalae and OFCs in the olfaction. In order to address several questions of monorhinal odor processing for functional pathways, [^{15}O]butanol-PET (**11**) was used to measure rCBF during monorhinic presentations of different odors (Figure 6).[58] Applying PET measurement of rCBF, it is able to investigate how the ipsilaterally entered olfactory signals can be processed, and also check whether right side dominace is independent of the stimulated nostril. Many studies showed that the olfactory dysfunction is a signal in the early stage of AD and PD. ^{18}F-FDG (**12**) is a well-characterized feature to image cortical hypometabolism in AD, which is belong to the AD-typical pattern of metabolic deficits. Using ^{18}F-FDG-PET combined with diffusion tensor imaging, researchers displayed the combination of fiber tract integrity in the olfactory tract with cortical glucose metabolism in subjects matching with mild cognitive impairment (MCI) and normal controls.[59] Ultimately, compared with normal controls, MCI showed diverse combination of olfactory tract integrity with functional metabolic changes. Later, olfactory tests with

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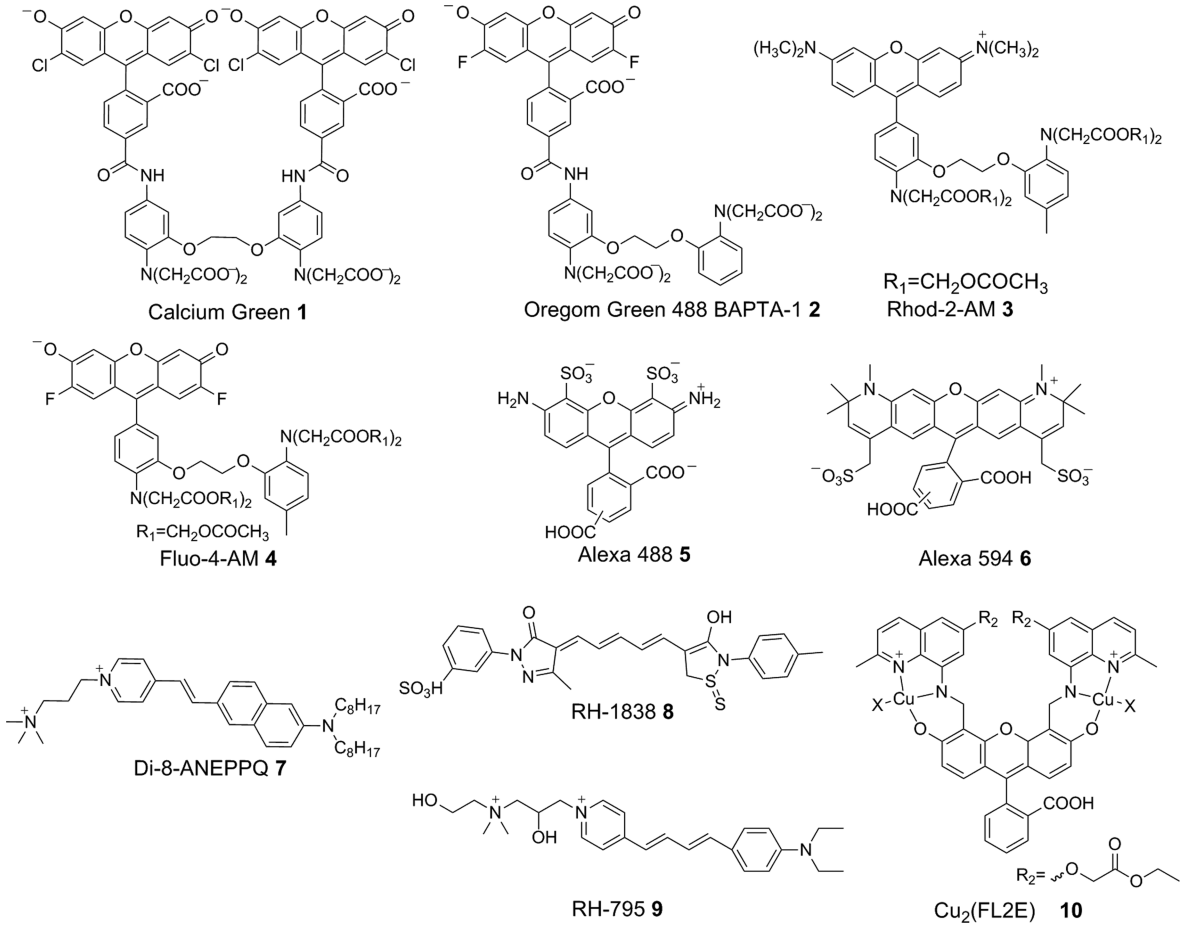

Figure 5. Structures of small molecular imaging probes for the olfactory system.

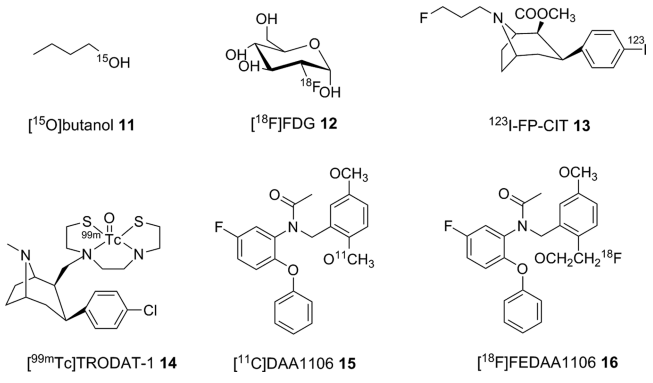

Figure 6. Nuclear medicine (PET/SPECT) probes for the olfactory system.

^{123}I-FP-CIT (**13**) can be applied SPECT imaging in clinical PD practice combined with transcranial sonography of the substantia nigra.[60] Additionally, the dopamine transporter [^{99m}Tc]TRODAT-1 (**14**) has been applied in the early PD study.[61] The peripheral benzodiazepine receptor (PBR) is a protein which is mainly distributed in OB and cerebellum. Different human pathology features, such as ischemia-reperfusion injury, neurodegenerative disease, and brain damage, are related to PBR that signify wide use in the future. Zhang and partners established a series of compounds in order to develop PET ligand for labeling PBR in the rat brain.[62] Later, in vitro and in vivo experiments suggested that [^{11}C]DAA1106 (**15**) and [^{18}F]FEDAA1106 (**16**) had higher binding affinities for PBR, higher uptakes, and higher in vivo specific bindings mainly in OB than previous reported PET probes. Among the brain regions examined, the highest radioactivity was observed in OB which has the richest PBR density in the rat brain.

4. IMAGING OLFACTORY SYSTEM WITH MRI PROBES

Although these PET or SPECT imaging probes are promising, certain drawbacks (especially the poor spatial resolution) restrict their clinical application. For MRI has many advantages including moderate cost-effectiveness (approximately one-fifth the cost of PET), high spatial resolution, probes of long storage period, and comparatively nontoxic labels, it makes MRI have a great impact on imaging olfactory system. Due to the high soft tissue resolution, MRI has superiority in investigating injuries of the brain soft tissue lesions than other imaging methods. It was reported that MRI is used to measure the volume of the OB and investigate the relationship probetween olfactory dysfunction and OB volume in subtypes of idiopathic PD.[63] Therefore, MRI has been employed to clarify the pathophysiology of posttraumatic olfactory dysfunction.[64] Notably, some MRI probes containing a contrast agent can also selectively target tissue and label a specific molecular entity into it. The transplantation of Schwann cells (SC) and ensheathing cells (OEC) has been confirmed to facilitate CNS axonal regeneration and remyelination.[65] Although many approaches have been developed for in vivo labeling of cell fate, only MRI has the resolution required to accurately localize transplanted cells in adult animals at present. Dunning and co-workers used

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SPIO nanoparticles to image olfactory SC and OEC of Fischer rat pups under MRI.[66] They demonstrated that these labeled cells can myelinate normally transplantation into central regions of demyelination. Except the simple paramagnetic metals as MRI tracers, the recent work led to a number of bioengineered probes for MRI. For example, conjugating gadolinium-tetraazacyclododecanetetraacetic acid (Gd-DOTA) with the cholera toxin B subunit (CTB) as the MRI tracer, Wu and colleagues disclosed a high signal in the OB labeled olfactory regions of the cortex.[67]

Manganese-enhanced MRI (MEMRI) is one of the unique methods for axonal transport assessments in a noninvasive manner. It is used to identify activity-sensitive neural in the olfactory system in the awaked rodent.[68] It is based on the accumulation of paramagnetic Mn^{2+} which can pass through the blood-brain barrier (BBB) and enter active neurons via voltage-gated calcium channels, even transport to adjacent neurons.[69] Recently, Chuang and partners conducted MEMRI to generate maps of neural circuitry based on the uptake activity and trans-synaptic transport of Mn^{2+}. They found that Mn^{2+} was delivered into the nasal cavity of mice and taken up by activated OSNs under exposure of the mice to defined odorants.[70] In addition, they confirmed that individual glomeruli can be detected by the Mn^{2+} mapping technique after comparing with images of GFP-labeled glomeruli. Besides, using MEMRI in rTg4510 transgenic mice, the presence of age-dependent axonal transport deficits was identified to begin at three months of age.[71] Researchers verified that it was possible to survey the change of signal intensity in the ONL of OB.

fMRI has also been widely used for studying neural processing, including olfactory processing. Currently, there are mainly two technologies in imaging OB of anesthetized rats under odor sensation: blood oxygenation level-dependent (BOLD) and cerebral blood volume weighted (CBVw) fMRI with iron oxide nanoparticles.[72] BOLD fMRI had the greatest activation on the bulb surface, midline, olfactory nerve, and glomerular layers, while CBVw activation had a higher sensitivity compared with BOLD fMRI which peaked in glomerular and external plexiform layers. In other words, it suggested that CBVw activation represented the visualization of metabolic activity in the OB better than BOLD does. However, BOLD fMRI can also differ between individuals with idiopathic environmental intolerance (IEI) attributed to odors and controls when exposed to low levels of olfactory and trigeminal stimuli. It was reported that the IEI group had higher BOLD fMRI signal than controls in the thalamus and a number of parietal areas, and lower BOLD signal in the superior frontal gyrus.[73] The development and application of BOLD fMRI imaging in awake mice was first reported to identify differences of brain activity affected by the odor of almond between wild-type, HETzQ175, and HOMzQ175 knock-in mice. Some data showed that the glomerulus of OB had decreased BOLD signal intensity changed by almond odor suggesting a deficit in olfactory sensitivity compared to the other genotypes in HOMzQ175 mice.[74] Additionally, using SPIO as the contrast agent and isoamyl-acetate as the odor stimulant, CBV fMRI was applied to investigate the odorant-induced olfaction in rhesus monkeys.[75] The stable odorant-induced CBV responses during three different stimulation periods were observed in the OB and suggested that the OB may not supply an important role in olfactory adaptation to odor. However, the applied technical approach can enable more extensive fMRI studies of olfactory processing in OB of both humans and nonhuman primates.

A study used fMRI successfully to probe the attentional modulation mechanism in primary olfactory cortex (POC) of human. And they highlighted the role of attention at the earliest cortical levels of olfactory processing.[76] To explore whether olfactory loss leads to deficits in olfactory imaging, fMRI and self-report measures were used to investigate in 16 participants and 19 control participants with acquired olfactory loss. Results from fMRI revealed that activation patterns differed between patients and controls. The results also indicated that participants with olfactory loss have difficulties to perform olfactory imaging in the conventional way.[77] In a clinical case, performing fMRI in anosmic patients with long-term smell loss can also investigate modifications of functional connectivity caused by olfactory training in the left and right piriform cortices.[78] The data introduced that olfactory training is able to induce changes in functional connectivity networks. Undergoing an fMRI investigation, a recent subject in healthy volunteers was developed to characterize the in vivo action of the putative human pheromone receptor VN1R1 expressed in the human olfactory mucosa. Then, it indicated that VN1R1 is involved in extraolfactory neuronal activations induced by Hedione. The activation of VN1R1 revealed that VN1R1 might play a key role in gender-specific modulation of hormonal secretion in humans.[79] Otherwise, the application of fMRI in olfactory system also appeared at a study of patients with Panic Disorder. In this study, a pattern of 13 patients with panic disorder and 13 healthy controls underwent an fMRI investigation during olfactory stimulation with their stress-related sweat odors which would influence the attentional focus and control odors as nonfearful nonbody odors. It demonstrated that the two groups did not differ according to their olfactory identification ability and indicated the results that altered neuronal processing of olfactory stimuli are in PD.[80]

5. CONCLUSION AND PERSPECTIVE

Molecular imaging of olfaction has undergone a dynamic evolution over the past decade. Up to now, many interesting studies have converged to show a great deal of molecular imaging technologies for monitoring the olfactory system. Following multimodal imaging studies including fluorescence, PET, and MRI imaging, several new biomarkers are being developed to image the olfactory process. These biomarkers for multimodality imaging could address the complex set of features required to make possibility for imaging olfactory in the clinical practice (Table 3). Molecular imaging has played a key role in studying the activation of olfactory system and could help to prevent or delay diseases that associated with olfactory disorders as a potent tool.[81] It is sure that molecular imaging has an ever-increasing contribution in reaching this goal by contributing to translational path physiopathologic research, drug development and early clinical diagnosis. Interestingly, we found that mice might be easily trained to detect target odorants embedded in unpredictable and variable mixtures. This superiority makes the rodent model to be the prime choice of studying the mammal olfactory activation. Although many imaging probes and media have been examined in olfaction, the mechanism involved in coding of odor information needs more trials to reveal. Additionally, the potentiality has not been fully realized by the pharmaceutical and imaging industries yet. We have strong belief that the more specific targeting probes with the noninvasive superiority should be a significant direction on imaging olfactory system in the future. As expected, the probe will play a key role for the

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Table 3. Multimodality Imaging of Biomarkers in Olfactory System

imaging modality	biomarkers	expressing approaches
fluorescent	Ca^{2+} signal	genetically encoded proteins
		Ca^{2+}-sensitive dyes
	voltage activities	voltage-sensitive dyes
	NO	NO-reactive probes
	olfactory sensory neurons	SpH
PET	peripheral benzodiazepine receptor	[^{11}C]DAA1106, [^{18}F] FEDAA1106
	metabolic activity	^{18}F-FDG
SPECT	pathological changes	^{123}I-FP-CIT
		[^{99m}Tc]TRODAT-1
MRI	metabolic activity	manganese-enhanced MRI
		CBVw fMRI
		BOLD fMRI

deep discovery of olfactory and introduction into daily clinical practice. As the use of selected probes allowed efficient and targeted olfactory condition imaging, it was considered that conjugating a therapeutic aspect into diagnosis could point out personalized treatment protocols and might improve prognoses versus standard treatment. This growing field has been known as "theranostics", which is a promising area in developing research in olfaction with applying molecular imaging, although there are many problems to be investigated and lots of work that should be done.[82]

No doubt, olfactory imaging is becoming reality according to the probes of different imaging modalities, such as PET, MRI, and optical imaging, in normal and diseased models of molecular activity. Thus, a number of receptors, transporters, and extracellular enzymes which relate to olfaction activity are potential targets for olfactory imaging. For example, spH, an indicator of neurotransmitter release, presents the neuronal activity when targeted to mature OSNs. Producing an exogenous spH as a novel fluorescent probe may be a bright approach to the olfactory system. OMP is a protein which selectively and highly expressed in mature OSNs. If combining with other fluorescent dyes, OMP could act an important signal to image the olfaction activity. On the basis of this design, by changing the imaging modalities such as PET, SPECT, and MRI, various tool molecules ready for olfactory detection can be constructed. Moreover, building a nanostructure with OMP as one targeting molecule also may become an insight direction. Accordingly, development of olfaction probes is a valuable field. The ultimate expected olfactory probes can localize and quantify noninvasively the potential targets activated in olfaction transduction process. Except the above potential targets, the combination of the two kinds of valuable dyes, calcium-sensitive dyes and voltage-sensitive dyes, may produce a new kind of dye in the future. This novel field will be applied better to image the activation of olfactory system for merging the advantages of the calcium-sensitive dyes and voltage-sensitive dyes together. The ideal design should have advantages of imaging both Ca^{2+} signal and voltage activities. Thus, with two targeting signals of olfactory activities, the designed probes will have high specificity and sensitivity in living organisms. As a result, further efforts and emphases should be placed on probes that have highly specific responses to the combinations of several biomarkers. And multidisciplinary integration for exploiting new identified mechanisms should play a key role in the future work.

What's more, the probes should obtain foresight into the molecular pathophysiology of olfactory diseases, and speed up the improvement of novel treatment.[83] For example, the imaging of olfactory function injury will play an important role in early diagnosis for AD and PD in the clinical setting. This suggests technological innovations of interdisciplinary research which generate various new constructs include neuropharmacology, biochemistry, systems biology, and so on. So it will be a valuable direction applying various novel concepts to design imaging probes specially targeting new signals in the circuit of olfaction. For instance, systems biology is now extended in its scope to identify metabolic networks, cellular signaling networks that explicitly include chemical molecules, and transcriptional and epigenetic networks to elucidate their functions and roles in human health and diseases. And systems biology is a potential useful theory for deeper research of the olfactory system relating to its processing and signaling.[84] These powerful subjects will offer valuable opportunities to study and image the biological processes of the olfactory system at the wider range and various levels.

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Author Contributions

W. Z. designed the review and directed its implementation. X. Z. contributed to the writing and review of the manuscript, and contributed to the literature search. A. B. wrote the manuscript and contributed to the literature search. Q. D. and S. Z. contributed to the review and revision of this manuscript. K. H. and Z. L. analyzed the data. T. G. contributed to the modification of the figures. All authors read and approved the final version of the manuscript.

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Notes

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ABBREVIATIONS

OB, olfactory bulb; ORN, olfactory receptor neurons; ONL, olfactory nerve layer; GL, glomerular layer; OSNs, olfactory sensory neurons; OE, olfactory epithelium; PD, Parkinson's disease; AD, Alzheimer's disease; PET, position emission tomography; MRI, magnetic resonance imaging; YFP, yellow emitting fluorescent proteins; GFP, green fluorescent proteins; spH, synapto-pHluorin; OMP, olfactory marker protein; SPECT, single photon emission tomographic; BOLD, blood oxygenation level-dependent

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