Olfactory Optogenetics

Subjects: Engineering, Biomedical

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The mammalian olfactory system has an amazing ability to distinguish thousands of odorant molecules at the trace level. Scientists have made great achievements on revealing the olfactory sensing mechanisms in decades; even though many issues need addressing. Optogenetics provides a novel technical approach to solve this dilemma by utilizing light to illuminate specific part of the olfactory system; which can be used in all corners of the olfactory system for revealing the olfactory mechanism.

optogenetics olfactory chemical sensing neuronal light

1. Introduction

As one of the oldest sensory systems, the mammalian olfactory system is capable of recognizing thousands of different odorant molecules, which can help creatures avoiding danger, looking for food, identifying spouses. The olfactory system has evolved a mature and perfect odor information processing mechanism. Odorant molecules are firstly sensed by olfactory sensory neurons (OSNs) of the olfactory epithelium (OE), where odorant receptors (ORs) are expressed in OSNs and interact specifically with odorant molecules. An OSN expresses only one type of OR protein, which belongs to the superfamily of G protein-coupled receptors (GPCRs). The specific interactions of ORs and odorant molecules cause OSNs to generate an electrical signal, which can be transmitted to the glomerular layer of the OB. The odor information is then projected by the mitral/cluster (M/T) cells of the OB through the lateral olfactory tract into the olfactory cortex (OC), including the anterior olfactory nucleus (AON), the piriform cortex (PC), the amygdaloid cortex (AOC), the olfactory tubercle (OT), and the lateral entorhinal cortex (LEC) [1]. At present, there is a general understanding of the structure of the olfactory system. However, the detailed mechanism of olfactory system requires further exploration. Much progress has been made in exploring the olfactory information processing mechanism, which mainly focuses on the structure and function of ORs [2][3][4], internal neural circuits of the OB [5][6][7], and feedback and centrifugal modulation of the OB [8][9][10]. The research progress of olfactory coding has been reviewed by many excellent articles [11][12]. OB transmits sensory input from OE to the OC and is modulated by intrabulbar circuits and centrifugal inputs. Therefore, it is a very necessary and difficult task to understand how different circuits mediate the various aspects of odor information encoding in OB.

How to deliver accurate odor stimuli to sensory cells has been a key technical challenge for investigating the olfactory system for decades. The emergence of optogenetic technology provides a novel and promising tool for this challenge [13][14]. The principle of optogenetics is to use light to control genetically engineered neuron populations with millisecond precision to activate or silence cells. The most commonly used optogenetics probes include depolarization and hyperpolarization genetics tools, such as channelrhodopsin-2 (ChR2) and *Natromonas* *pharaonis* halorhodopsin (NpHR) [15][16]. Some more flexible and refined tools have been expanded, such as ChETA [17] and ReaChR [18], which can activate target neurons at a higher frequency. Optogenetics can be used to elucidate neural circuit activity by controlling specific neuronal populations, which has ushered in important breakthroughs in the field of neuroscience. At the same time, optogenetic technology has also been used to reveal the mysteries of the olfactory system. Some optogenetic transgenic animal models have been developed for the research on the chemical sensing mechanisms of biological olfactory system, and are illustrated in **Figure 1**. Light–sensitive proteins have been expressed in a variety of neurons in the olfactory system, including OSNs in the OE, main output neurons and inhibitory interneurons in the OB, neurons in the OC, and neurons in the neuromodulation system dominating the OB. Thanks to its relationship between mammals and humans, as well as its technological maturity and expansion, the applications of optogenetics in non-human primates have also progressed, focusing on the primary motor cortex (M1) [19] or the frontal eye field (FEF) [20].

Interestingly, the precise light stimulation can replace the unstable odor delivery when necessary, making optogenetic technology extremely attractive in olfaction research. In recent years, optogenetics has been extensively applied in many fields, and some excellent reviews have summarized the progress of the application of optogenetics in the research of hippocampus related to memory [21], the prefrontal cortex associated with cognition [22], the amygdala associated with pain and anxiety [23], and neurological disorders such as depression and psychosis [24]. However, although optogenetics has been widely applied in the research of the olfactory sensing mechanism, the important applications of optogenetics in the olfactory system have rarely been outlined and discussed. Grimaud et al., reviewed the contributions of optogenetics in the study of olfactory learning and memory, but in recent years, scientists have made many outstanding advances in olfactory research using optogenetics [25].

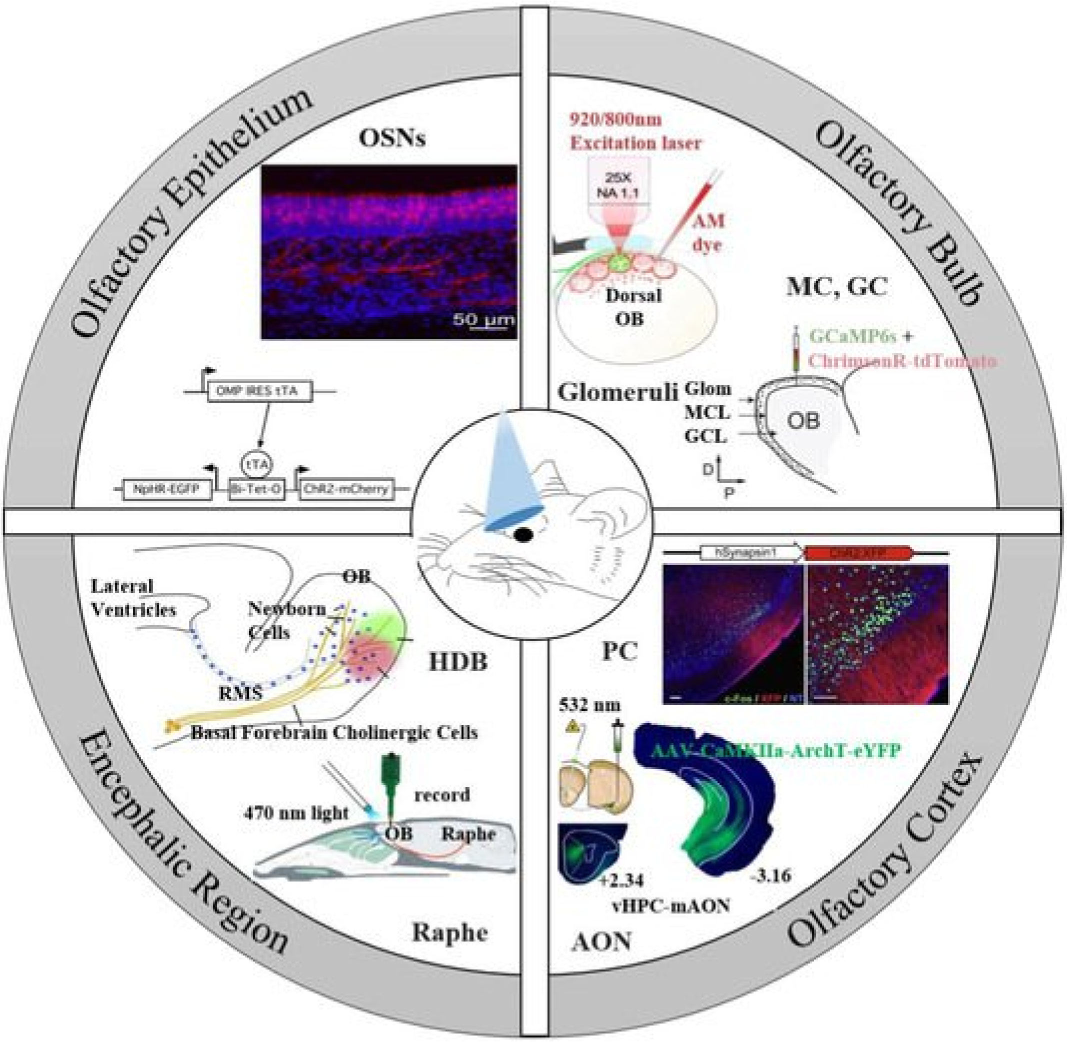

Figure 1. Application of optogenetics in various parts of the olfactory system. Olfactory epithelium: ChR2 is expressed in all OSNs to test whether mice can perceive the time of olfactory stimulation [26]. Olfactory bulb: ChR2 was expressed in a single glomerulus to study its response to optogenetic stimulation [27]. Express ChrimsonR-tdTomato in mitral cells and granule cells of mice to explore the coding characteristics of its perception detection [28]. Olfactory cortex: explore whether light-activated piriform cortex neurons of different subtypes can induce different behaviors [29]. Archaerhodopsin is used to inhibit the hippocampus dominating the AON subregions, revealing the principle of odor memory [30]. Encephalic region: ChR2 was expressed in the dorsal raphe nucleus to study the olfactory regulation of serotonin [31]. Express ChR2 in HDB and explore the olfactory perceptual learning involving cholinergic neurons [32]. Reproduced with permission from [26], Copyright 2012 Society for Neuroscience [27], Copyright 2018 Springer Nature [28], Copyright 2020 Elsevier [29], Copyright 2011 Elsevier [30], Copyright 2018 Springer Nature [31], Copyright 2016 Society for Neuroscience [32], Copyright 2019 Elsevier.

2. Optogenetic Tools for the Olfactory System

Optogenetic tools include dozens of light-sensitive proteins, which can be activated by different wavelengths of light with various operating speeds. Microbial rhodopsins undergo membrane depolarization or cellular signaling cascades caused by light-induced photochemical reactions [33], and are widely used in neuroscience, including the olfactory system. Channelrhodopsin-2 (ChR2) was the first optogenetic tool to activate neurons with light, and is the most commonly used one due to its rapid on-rate [16]. When ChR2-expressed neurons are illuminated by light with specific wavelength (450–490 nm), neurons will be depolarized by activating channels [14]. These light-sensitive proteins such as ChR2 could be introduced to olfactory neurons by a viral vector or transgenic animals. Under the driving of various promoters, adeno-associated viruses (AAVs) are used to target ChR2 in M/T cells, interneurons in OB, cells in AON, and PC [34][35][36][37]. Lentiviral vectors (LVs) are also used to target ChR2 in the rostral migratory stream (RMS) and PC [29][38]. Optogenetic tools can be guided to the nervous system through a viral expression system, and targeted neurons with the help of specific promoters are summarized in **Table 1** and **Table 2**. Specific promoters drive light-sensitive proteins to target different neurons, such as Pcdh21 targeting M/T cells, TH targeting short-axon cells, CHR targeting interneurons in the EPL, and choline acetyltransferase promoter targeting choline acetyltransferase neurons [34][39][40][41]. This genetic technology enables specific types of neurons of the olfactory system to be activated or silenced by light in order to study the role and functional connection of specific neurons in the olfactory system.

Several transgenic animal lines have been created for the research of the olfactory system. Arenkiel et al., generated transgenic mice expressing ChR2-YFP from the Thy1 promoter for the precise and rapid activation of mitral cells in the OB [42]. Then, they used Thy1-ChR2-YFP mice for mitral-cell-specific light stimulation to map the functional connectivity between mitral cells and interneurons [40]. They also generated VGAT-ChR2 transgenic mice with ChR2-expressed GABAergic neurons and glycinergic inhibitory neurons to study the neuronal connectivity [43]. Additionally, Dhawale et al., generated transgenic mice by expressing ChR2-EYFP into OSNs and their axons with a promoter OMP [44]. In generated transgenic mice, the individual M/T cell can be activated by illuminating a single glomerulus. OMP-ChR2-YEP transgenic mice and M72-ChR2 transgenic mice lines were created to study olfactory perception (details in Section 4) [45][46]. In summary, these ChR2 transgenic animals allow scientists to selectively activate olfactory neurons and study the signal processing and perception in olfactory system [47].

Gunaydin and colleagues designed and verified the E123T mutation in ChR2 (ChETA) to address the precision limitations of ChR2 [48]. The Cre-dependent AAV-ChETA-EYFP vector was injected into the OT for cell-type-specific expression to study the roles of OT neurons in attractive and aversive behaviors [49]. Similarly, Aqrabawi and colleagues used AAV-ChETA-eYFP vectors to express ChETA-eYFP in AON neurons in FosCreER mice to manipulate populations of neurons in AON which constitute odor engrams [50]. Lin and colleagues engineered a variant of channelrhodopsins named denoted red-activatable ChR (ReaChR), which enables transcranial optical activation of neurons [51]. Using the novel optogenetic tool, Inagaki and colleague generated the UAS-ReaChR transgenic Drosophila for neural manipulation [52]. This transgenic model has helped to understand the neuronal functions of Drosophila mushroom body (a higher olfactory circuit) and the sensitivity regulation in insect primary olfactory neurons [53][54].

The proton pump archaerhodopsin (Arch) from *Halorubrum sodomense* or the archaerhodopsin from the Halorubrum strain TP009 (ArchT) were used as optogenetic neuronal silencing tools. To selectively silence GABAergic inhibitory neurons, scientists injected AAV-ArchT virus into granule cell layers in the OB of transgenic mice expressing Cre recombinase. This optogenetic technology, combined with intracellular recordings, examined the contribution of inhibition to rhythmic activity in the mouse olfactory bulb [55]. McCarthy and colleagues injected AAV-ArchT virus into the accessory OB of Pcdh21-Cre mice in which expression of Cre-recombinase is restricted to M/T cells to examine whether light inhibition of accessory OB neurons could affect lordosis in sexually mice [34]. In addition, another commonly used silencing tool is *Natronomonas pharaonis* halorhodopsin (NpHR). The modified NPHR is called eNpHR3.0, which has better targeting of cell membrane, a longer current, shorter response time, and more sensitive response. The pLenti-hSyn-eNpHR3.0-EYFP lentivirus [56] were injected in the OB to study the OB projection to the olfactory tubercle [57] and to examine the effect of anterior OB inhibition on odorant attraction [58].

Table 1. Optogenetics approach in interneurons in OB.

Level	Expression Target	Model Animal	Expression Approach	Light Delivery					Electrophysiology Recordings	Behavior	Ref.
				Tool	Wavelength (nm)	Duration (ms)	Frequency (Hz)	Power (mW)			
GL	Glomeruli	OMP-ChR2-YFP transgenic mice	Transgenic animal model	A 470 nm LED coupled with an objective	470	10	-	-	M/T cells: patch-clamp	-	[6]
	SACs	TH-Cre mice	Injection of AAV-ChR2 into the GL	A solid-state laser coupled with an optical fiber	473	-	-	100	M/T cells or ETCs: whole-cell patch-clamp or cell-attached and tungsten microelectrodes	-	[39]
EPL	EPL-INs	Crh-Cre mice	Injection of AAV-ChR2 into the OB	A BLM-Series 473 nm blue laser system coupled	473			20–40	EPL INs: whole-cell patch-clamp	Olfactory associative learning training	[40]

Level	Expression Target	Model Animal	Expression Approach	Light Delivery					Electrophysiology Recordings	Behavior	Ref.
				Tool	Wavelength (nm)	Duration (ms)	Frequency (Hz)	Power (mW)			
				with an objective							
	EPL-INs	CRH-Cre mice	Injection of AAV-ChR2 into the OB	A blue laser system guided by implanted fiber optics	473	10	-	30	MCs: whole-cell patch-clamp and extracellular recording electrodes	Olfactory associative learning training	[59]
IPL	dSACs	Chrna2-Cre mice	Injection of AAV-ChR2 into the IPL	A 75 W xenon arc lamp coupled with an objective	-	-	-	-	TCs: whole cell patch clamp	-	[35]
GCL	GCs	Dlx5/6-Cre mice	Injection of AAV-ChR2 into the OB	A BLM-Series 473 nm blue laser system coupled with an objective	473			20–40	GCs: whole-cell patch-clamp	Olfactory associative learning training	[40]

Encephalic Region	Expression Target	Model Animal	Expression Approach	Light Delivery					Electrophysiology Recordings	Ref.
				Tool	Wavelength (nm)	Duration (ms)	Frequency (Hz)	Power (mW)		
Basal forebrain	HDB cholinergic neurons	VGLUT3-Cre mice	Injection of AAV-ChR2 into the HDB	A 75W xenon arc lamp coupled with an objective	-	10–20	-	-	OB cells: patch clamp	[61]

Encephalic Region	Expression Target	Model Animal	Expression Approach	Light Delivery					Electrophysiology Recordings	Ref.
				Tool	Wavelength (nm)	Duration (ms)	Frequency (Hz)	Power (mW)		
		ChAT-ChR2-EYFP transgenic mice	Transgenic animal model	A diode-pumped solid-state 473 nm laser coupled with an optical fiber target the HDB	473	15	5–50	-	M/T cells and brain slices: patch clamp	[41]
		ChAT-ChR2-EYFP mice transgenic	Transgenic animal model	A blue light diode laser and a blue LED coupled with implanted fiber	473	15	5–50	-	-	[32]
	HDB GABAergic neurons	DLX5/6-Cre mice	Injection of AAV-ChR2 into the HDB	A blueCoolLED pE 100 coupled with an objective	490	-	-	-	OB cells: whole-cell	[62]
		ChAT/GAD2-Cre mice	Injection of AAV-ChR2/eNpHR into the HDB	A 470 or 565 nm LED coupled with an optical fiber positioned in the OB	470; 565	10000	-	10;3	M/T cells: sixteen channel electrodes	[63]

Encephalic Region	Expression Target	Model Animal	Expression Approach	Light Delivery					Electrophysiology Recordings	Ref.
				Tool	Wavelength (nm)	Duration (ms)	Frequency (Hz)	Power (mW)		
Raphe nuclei	5-HT axons	TPH2-ChR2-YFP transgenic mice	Transgenic animal model	A bright light-emitting diode (LED) array coupled with a microscope	473	10	10	15	M/T cells: tungsten electrodes and whole-cell	[64]
	serotonergic cells	Slc6a4-Cre mice	Injection of AAV-ChR2 into DRN	A 470 nm LED coupled with a glass fiber positioned close to the OB	470	10000		1–10	OB cells: 16-channel electrode	[31]
	serotonergic cells	SERT-Cre mice	Injection of AAV-ChR2 in the DRN	A 470 nm laser coupled with an optrode lowered into the DRN	470	10	1–30	-	APC neurons: microelectrodes;	[65]
locus coreuleus	noradrenergic neurons	DBH-Cre-NpHR transgenic mice	Transgenic animal model	A solid-state laser coupled with an optical fiber implanted in the OB	532	-	-	2–10	MCs: tetrodes	[66]

[68] [69] [70][71] [72] [26] [73][74]

rmation. e OB [67] l signals ssed the onses of e use of of odor he ChR2 rol of the gene to wed that and the hat MCs SNs was m OSNs ssing the on in the edundant

information was worth pondering. Albeanu et al., determined the M/T sister cells via the light-activated electrophysiological signals of a single glomerulus [44]. Transgenic mice expressing ChR2-EYFP in OSNs were used in this study to enable the glomerular layer (GL) to be accurately activated by light, while extracellular recordings showed that M/T cells only responded to the light stimulation of the GL. Because the size of the light spot was close to the average size of a single glomerulus, a single glomerulus can be activated for recording the response of M/T cells, which allowed the correct identification of the parental glomerulus to each mitral/tufted unit, and was further used as a basis to divide M/T cells into sister cells and non-sister cells. Studying the odor responses of sister cells found that the changes in their firing rates were related, indicating that sister cells received a common excitatory input. Moreover, the non-redundancy was found in the temporal characteristics of sister cell activity, which was of great significance for the transmission of information from the OB to the cerebral cortex.

In short, in the process of transmitting odor information to the OB, OSNs, glomeruli, MCs, and TCs played different roles to complete this task, respectively. After the direct OSN-EPSCs arrived at the TC, the MCs received a strong multi-step signal through the TC. Meanwhile, although a pair of sister cells (M/T) receive a common glomerular input, there are non-redundant odor responses. With the help of optogenetics, the goal of precise control of OSNs or a single glomerulus can be achieved, and then the downstream neural response can be measured to analyze the projection relationship with M/T cells.

4. How Do Activities of the Olfactory Bulb Neurons Affect Perception?

An important issue in olfactory research is how the brain encodes and processes odor information, i.e., how to convert chemical perception signals stimulated by odors into complex brain activities and behaviors. Imaging, electrophysiology, pharmacology, genetics, and other methods were used to reveal the basic principles of olfactory codes, including phase coding [75], combined coding [76], sparse coding [27], and concentration coding [77]. More studies on olfactory coding have been carefully discussed in some excellent reviews [11][78][79], and this review focuses more on the role of optogenetics in it. Light can selectively activate glomeruli instead of odor stimuli, and the perceptual responses can be explored by adjusting different characteristics of light stimulation, including the start and end time, the duration, the population, and number of glomeruli of light stimulation, etc. (**Figure 2**). As a result, odor perception is associated with complex neural activity patterns, and optogenetics is used as a powerful weapon to unlock the mystery of olfactory encoding.

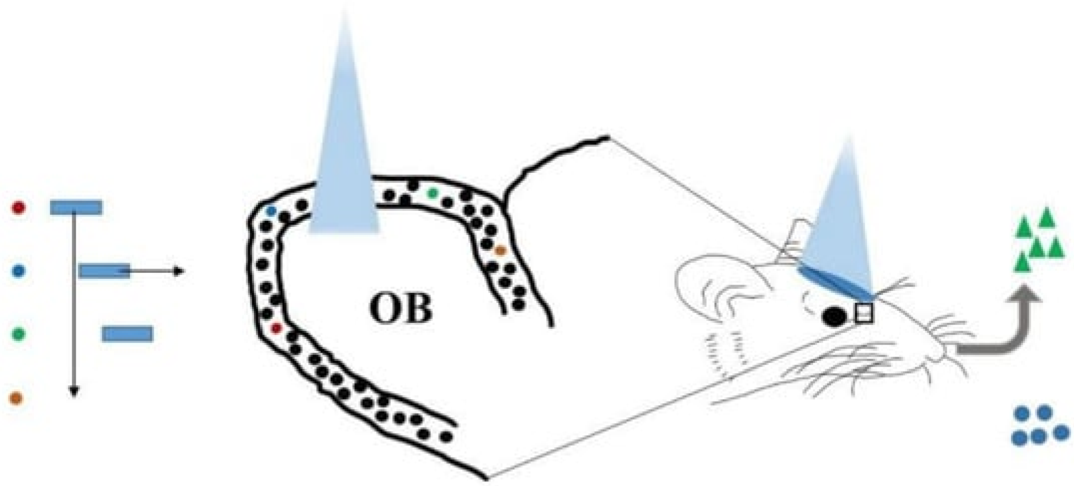

Figure 2. A general strategy for exploring the link between brain activity and olfactory perception using optogenetics. Optogenetics is used to manipulate the spatiotemporal patterns of glomerular or M/T cell activity (such as activation population, latency, duration, etc.), followed by behavioral measurement of animal olfactory discrimination and perceptual limits.

The timing of odor stimulation is a key parameter and may represent important information of the olfactory system. In order to better understand the role of stimulus timing in the olfactory system, and to explore whether and how animals read the time of olfactory activation relative to the sniff cycle ('sniff phase'), Rinberg et al., from the New York University Neuroscience Institute used optogenetics to generate time-controllable light stimulation to activate olfactory sensory neurons [45]. The results of behavioral and electrophysiological recordings indicated that the mouse's olfactory system can accurately detect the beginning of the stimulus relative to the olfactory cycle and read out the time pattern. However, another group of researchers from the John B. Pierce Laboratory used the same method to prove that mice can successfully distinguish the onset time and delay of virtual odor stimulation, regardless of sniffing [80]. The opposite result did not negate the role of the sniffing cycle but indicated that the internal glomerular timing (the activation time relative to other neurons in the sequence) also participated in the encoding of time information, thereby independently affecting the perception of smell. Animals can not only perceive the beginning and end of light stimulus, but also distinguish the duration of the stimulus. Li et al., stimulated ChR2-expressed OSNs of mice at different durations [81]. Animal behavior results showed that mice could distinguish stimuli with a duration of 10 milliseconds, demonstrating that the mammalian olfactory system could accurately sense the difference in odor input duration. They also explored the thorny issue of neuron processing of input duration. The results of electrophysiological records showed that, when the glomeruli were activated by light of different durations, M/T cells responded strongly, and the frequency of their stimulation spikes carried the information of the stimulation duration.

Using time-controllable precise light stimuli instead of odor stimuli, scientists discovered that the characteristics of the time activity can impact on olfactory perception, including the start time, end time, and duration of the stimulus. In addition, the neural activity that induces perception should also include some spatial characteristics, such as what cells are responding to and the impact of the response of a single cell or multiple cells on cognition. Rinberg's group have made many striking breakthroughs on this subject using optogenetics technology in recent years. They first explored whether the mouse can perceive the activation of a single glomerulus [46]. The coding sequence of ChR2-YFP was inserted into the gene encoding the olfactory receptor M72 to activate individual glomeruli with light. By changing the intensity and duration of light stimulation and other parameters, it was found that a single glomerulus can convey odor information using intensity and time coding prompts. However, odor stimuli usually induced a group of glomerular responses, which not only varied with odor, but also varied with the concentration of a single odor. Their team proposed an odor coding scheme called "primary coding", which meant that some of the earliest activated glomeruli were very crucial for identifying odors [82]. In order to test this hypothesis, they delivered light stimuli to the ChR2 expressing OSN to generate masking stimuli. Combined with the electrophysiological recording and behavioral tests, they found that the earliest induced neural activity can be used to make olfactory judgments, confirming this olfactory primary code scheme.

Using optogenetic technology to explore the sense of smell, researchers can measure the limit of behavior discrimination by precisely manipulating a single feature (a single time or space feature), which confirmed the importance of the above single feature for olfactory perception. Thus, how will the combined characteristics of neuron spatiotemporal activities affect olfactory perception? Researchers performed optogenetic operations on mice to link the complex spatiotemporal activity patterns of neurons with olfactory perception. Chong et al., studied the importance of the combined characteristics of the spatial identity of glomerular activation and the temporal latency for perceptual meaning [83]. They used OMP-ChR2-YFP mice to manipulate the activated neuron groups or the activation latency with precise light stimulation, and then measured the changes in mouse cognition under different activity patterns. The results showed that activating different cell groups or changing their activation latency relative to other cells would cause varying degrees of changes in cognition. In this study, they did not explore the animal's perception limit, and a more advanced optogenetic technology would provide the possibility to solve this problem. The holographic two-photon optogenetic technology can selectively activate neuron collections with the single cell resolution [84], achieve the spike–scale stimulation with millisecond resolution via the latest soma-covering light spots technology [85], and reproduce the temporal and spatial resolution of olfactory neuron activity patterns. In the latest study, Rinberg et al., used a modified light stimulation to activate neurons expressing red-shifted opsin ChrimsonR, and explored the three stimulus characteristic dimensions of mice (i.e., number of response cells, synchrony between neurons, and latency from inhalation onset) [28]. Their latest results showed that mice can detect a single action potential synchronously evoked by less than 20 M/T cells. At the same time, the synchronization of activation between neurons affected the olfactory perception of mice more than the inhalation period.

Odor stimulation induces neuronal activity, and the spatio-temporal combination of glomerular activity corresponds to different odors and even their concentrations [86], so the temporal and spatial features of odor perception have extraordinary significance for odor decoding. Recent research has explored the influence of multi-dimensional features such as number, synchronization (relative to the inhalation phase or not), latency and duration of glomeruli, and M/T cells activation on olfactory perception. Dr. Rinberg's group has made outstanding contributions to the research on this topic. Although it may not completely replace natural odor stimulation, optogenetics provides precise parameterization and good control of causal operation with minimal damage, combines behavioral means to explore the contribution of these features to olfactory perception, and helps us better understand the basic principle of olfactory coding.

5. The Function of OB Interneurons in Odor Information Processing

The OB is the first relay station for odor information processing, connecting the sensory input of the OE with the olfactory area of the brain. The M/T cells in the OB receive odor information from the glomerulus, and the information is then transmitted to higher brain regions for further processing. M/T cells are modulated by various local interneurons in the OB during odor information processing. As shown in **Figure 3**, modulation involved neurons mainly include external tufted cells (ETCs), periglomerular cells (PGCs), superficial short-axon cells (sSACs) in the glomerular layer (GL), external plexiform layer interneurons (EPL-INs), deep short-axon cells (dSACs) in the internal plexiform layer (IPL), and granule cells (GCs) in the granule cells layer (GCL). Understanding the basic function of neuronal circuits in the OB may help decrypt the complex odor coding principles. Recently, optogenetics has been employed to reveal the complex modulation mechanism in OB. **Table 1** summarizes the application of optogenetics approaches in investing the function of OB interneurons. Similar to the section on the functional connection between OE and OB, the general strategy of optogenetics in OB interneurons is to manipulate the characteristics of neuronal activation (in this case, the type of cell) and then measure the response of the downstream neuron to explore the projection from one area to another, where it is mainly the regulation of various types of interneurons on the M/T cells of main cells in OB.

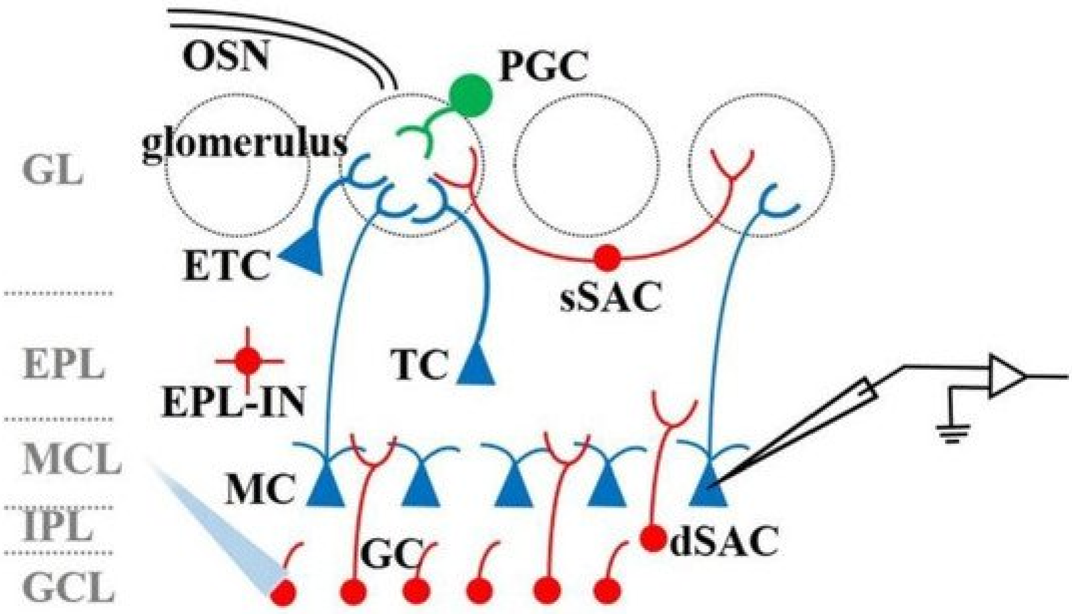

Figure 3. Schematic diagram of the structure of the OB. The method of using optogenetics to study interglomerular interactions is to activate specific interneurons, and then record the electrophysiological signals of M/T cells.

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