Molecules and Cells

Minireview

Advances in Optical Tools to Study Taste Sensation

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Taste sensation is the process of converting chemical identities in food into a neural code of the brain. Taste information is initially formed in the taste buds on the tongue, travels through the afferent gustatory nerves to the sensory ganglion neurons, and finally reaches the multiple taste centers of the brain. In the taste field, optical tools to observe cellular-level functions play a pivotal role in understanding how taste information is processed along a pathway. In this review, we introduce recent advances in the optical tools used to study the taste transduction pathways.

Keywords: geniculate ganglion, gustation, imaging, insular cortex, optical tools, taste bud

INTRODUCTION

Taste sensation is the process of converting chemical identities in food into a neural code of the brain. Taste information initially forms on the tongue when tasting molecules reach the taste receptors located on the apical microvilli of taste buds (Roper and Chaudhari, 2017) (Fig. 1). Taste buds can distinguish at least five basic tastes, sweet, umami, bitter, sour, and salty, using distinct sets of G protein-coupled receptors or ion channels (Ogata and Ohtubo, 2020; Shrestha and Lee, 2021; Zhang et al., 2003; Zhao et al., 2003). Taste information travels through the afferent gustatory nerves that innervate taste buds to the sensory ganglia positioned in the facial canal (i.e., the geniculate ganglion for the anterior tongue and the petrosal ganglion for the posterior tongue). Taste information is then transmitted to the nucleus of the solitary tract (NST) in the brain stem and further processed in multiple brain areas, which also integrate other sensory inputs and internal states (Schiff et al., 2018; Wang et al., 2018).

Optical tools have become pivotal in taste research because they provide a means to record cellular information in the natural environment. Various chemical- or protein-based fluorescent indicators have been developed to generate optical contrast for specific cellular phenomena (Lambert, 2019). Fluorescence microscopy readily provides millisecond-scale temporal dynamics with subcellular-scale spatial precision. The limited penetration depth of light into scattering biological media can also be overcome by minimally invasive surgical procedures tailored to specific organs of interest (Choi et al., 2015a). In this minireview, we discuss recent advances in optical tools adopted for studying the major areas of the taste transduction pathway, from the tongue to the brain.

TASTE BUD

Multiple experimental preparations for taste buds have been utilized to understand the initial steps of taste information processing. Early studies relied on *in vitro* preparations by enzymatically digesting the extracellular matrices and culturing isolated taste bud cells in Petri dishes (Ozdener and Rawson, 2013). Although beneficial for biochemical assays, isolated cell cultures largely compromise the intracellular interactions within the taste bud. To better preserve the intercellular architecture, *ex vivo* preparations, such as tongue epithelial

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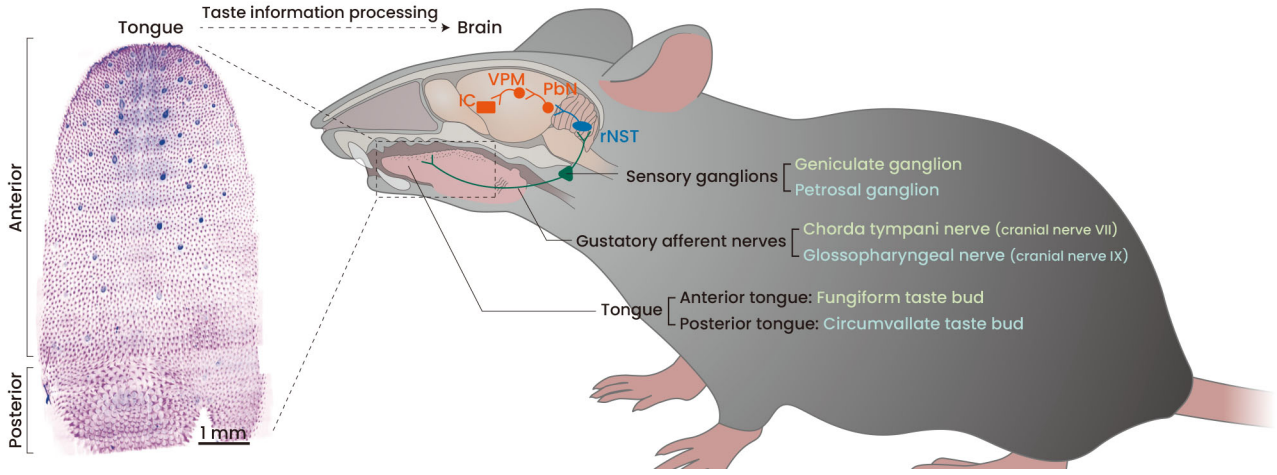

Fig. 1. The taste transduction pathway from the taste buds on the tongue to the insular cortex in the brain. The left image is a two-photon microscopic image of the mouse tongue with an inverted look-up table (magenta, autofluorescence; purple, second harmonic generation signal). In the schematic image on the right, the major areas responsible for taste information processing are illustrated. IC, insular cortex; VPM, ventral posteromedial nucleus; PbN, parabrachial nucleus; rNST, rostral nucleus of the solitary tract.

sheets and isolated taste buds, were used. The tongue sheet was prepared by peeling off the tongue epithelium after mucosal injection of dispase and collagenase (Venkatesan et al., 2016). Individual taste buds were further isolated from peeled epithelial sheets and affixed to a lysine-coated dish for functional studies, including electrophysiological recordings and fluorescent calcium imaging, in response to various gustatory stimuli (Ruiz et al., 2001). However, these preparations could not mimic the compartmentalized cytoarchitecture of the taste bud, in which only the apical microvilli are exposed to oral tastants, whereas the remaining cell bodies are shielded from the external environment by tight junctions at the apical tip.

Several preparations have been proposed to preserve the physiological compartments within taste buds. Yoshida et al. (2009) devised a setup for the isolated taste bud in which the mucosal side was drawn to a stimulating pipette perpetually perfused with Tyrode's solution, set apart from external tastant stimuli entering the apical microvilli. The taste stimuli were restricted to the taste pore, which was rinsed with distilled water between each stimulus. Electrophysiological recordings have shown that taste bud cells can be classified into cellular subtypes that respond to various tastants (Yoshida et al., 2009). Similarly, Richter et al. (2003) harnessed a method to activate taste bud cells on acute lingual slices by focally delivering tastants with a stimulus micropipette specifically on the apical pore. These methods successfully reproduced the natural microenvironment of the taste bud, where only the apical tip was exposed to premediated stimuli, while the basolateral side remained perfused within the epithelium. Such methods have led to the discovery that delivery of weak acids into the taste pore depolarizes a few taste receptor cells and induces an increase in intracellular calcium (Richter et al., 2003).

For functional imaging, taste bud cells must be stained with either chemical or genetic contrast agents. The most widely used functional indicator targets intracellular calcium, which serves as a second messenger for transducing the most basic taste qualities (e.g., sodium taste) (Nomura et al., 2020). The intracellular loading of chemical indicators can be achieved by *in vivo* iontophoresis (e.g., calcium-green-dextran) or bulk diffusion *in vitro* (Fura 2-AM) (DeFazio et al., 2006). Genetically encoded calcium indicators (e.g., GCaMP) can also be introduced using transgenic mouse lines with promoters active in taste bud cells such as *PIRT* (for chemosensory type II and III cells) and *GAD2* (for glia-like type I cells). Other types of functional indicators, such as pH indicators (e.g., BCECF-D), have also been used to study changes in intracellular pH in response to sour stimuli (Richter et al., 2003).

Calcium imaging has been commonly used to investigate taste-evoked activities of taste bud cells. Calcium imaging of a peeled tongue epithelium revealed that salt-sensing cells could be classified as ENaC-dependent sodium-sensitive cells and ENaC-independent high-salt-sensitive cells (Chandrashekar et al., 2010). Using a similar preparation, Oka et al. (2013) also showed that high salt concentrations activate bitter-sensing cells (and potentially sour-sensing cells) to mediate behavioral aversion to high salt. Caicedo and Roper (2001) used calcium imaging of a lingual slice preparation to demonstrate that different bitter compounds activate distinct subpopulations of bitter-sensing cells.

Calcium imaging was later modified to study specific neurotransmitter dynamics in taste buds. This approach involved the genetic engineering of extraneous cultured cells to express a specific neurotransmitter receptor and loading them with a calcium indicator (referred to as a "biosensor cell") (Huang et al., 2009). Using a fine micropipette, the biosensor cell was positioned near the target taste bud or cell to detect neurotransmitter release by observing cellular calcium dynamics. For instance, Chinese hamster ovary cells genetically engineered to express a purinergic receptor or a GABA

receptor can be loaded with a calcium-sensitive dye to screen extracellular ATP or GABA, respectively. The use of biosensor cells has led to the finding that stimulating type II and type III taste cells induce the release of ATP and GABA, respectively (Huang et al., 2009; 2011). Additionally, by observing ATP secretion from type II cells in response to sweet stimuli in circumvallate taste buds, adenosine has been shown to modulate the responsiveness of type II cells (Dando et al., 2012).

An intravital tongue imaging window was developed for the fungiform taste bud to fully recapitulate the living microenvironment of the taste bud (Choi et al., 2015b). In this preparation, the mouse tongue was externalized from the oral cavity using a miniature suction grabber and sandwiched in metal fixatives to minimize physiological motion. The anterior tongue was immersed in artificial saliva and imaged using a two-photon microscope with a water immersion objective lens. Using this preparation, Choi et al. (2015b) demonstrated that taste bud cells are responsive to both orally administered and circulating tastants (e.g., saccharine and NaCl) in the bloodstream.

The intravital imaging window was elaborated in due course by the integration of a microfluidic channel, named "μTongue (microfluidics-on-a-tongue)" (Han and Choi, 2018) (Fig. 2A). The μTongue permits programmable delivery of various tastants of interest topically on the anterior tongue to observe taste-induced functional activities of taste cells in real time (Han et al., 2021a). The imaging system was later improved by introducing an axially elongated beam (i.e., Bessel beam) into two-photon microscopy, which largely resolved the issue of the focal shift introduced by high-index tastant solutions, such as high concentrations of NaCl and sucrose (Han et al., 2021b).

SENSORY GANGLION

Sensory information from the tongue travels to the sensory ganglia located in the facial canal of the head (Fig. 1). As illustrated in Fig. 1, there are two relay stations: the geniculate ganglion innervating the fungiform taste buds in the anterior tongue and the petrosal ganglion innervating circumvallate taste buds in the posterior tongue. The most widely studied are the geniculate ganglion housing cell bodies of pseudounipolar sensory neurons receiving taste information through the chorda tympani nerve (Yokota and Bradley, 2016; 2017) and transmitting signals to NST in the brainstem and thalamus. Observing the neuronal population activity in the geniculate ganglion provides input gustatory information to the brain.

As neuronal firing is accompanied by an increase in intracellular calcium, the spatiotemporal activities of the geniculate neurons can be imaged using genetically encoded calcium indicators (e.g., GCaMP) (Lee et al., 2019; Wu and Dvoryanchikov, 2015). Previous studies used transgenic reporter mice with pan-neuronal promoters such as Thy1-GcaMP6 and SNAP25-GcaMP6. An alternative approach is the viral delivery of genetically encoded calcium indicators by injecting retrograde viral vectors into the NST, where geniculate neurons project and form synapses (Yarmolinsky et al., 2009).

Once the animal model is prepared, the geniculate ganglion must be surgically exposed to ensure optical access. The geniculate ganglion can be reached via either the ventral or dorsal routes, which reveal intact geniculate neurons without perturbing the connected nerve fibers (Fowler and Macpherson, 2021; Lundy and Contreras, 1999; Wu and Dvoryanchikov, 2015; Yokota and Bradley, 2016). Tracheotomy is often performed to ensure adequate physiological respiration. As geniculate neurons lie deep within the facial canal, the imaging system must have an adequate working distance of >1 cm. Typically, widefield or confocal fluorescence microscopes with long working distance objective lenses (e.g., 10X, 0.3 NA, 16 mm working distance) are used. A narrow microendoscopic probe consisting of a gradient index (GRIN) lens was also developed as an alternative, allowing better spatial resolution with a higher numerical aperture (Fig. 2B). Time-series images of the geniculate neurons can be recorded in response to various tastants delivered to the anterior

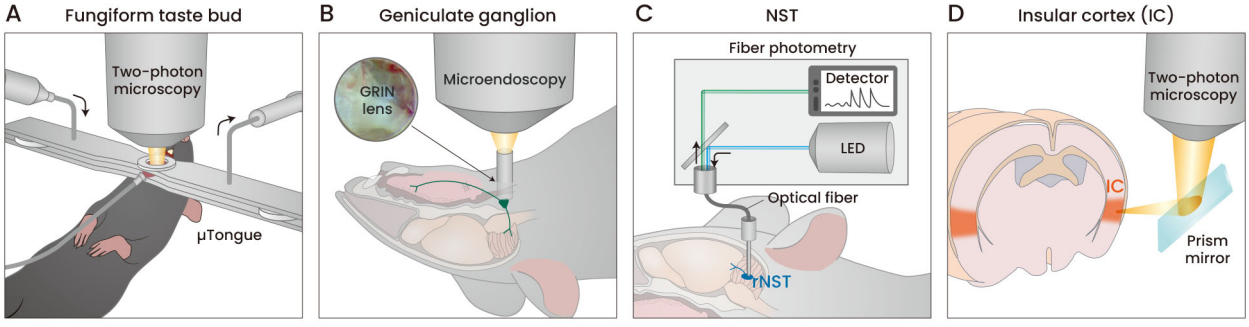

Fig. 2. Representative optical techniques used for studying the taste transduction pathway. (A) Microfluidics-integrated imaging window for the anterior tongue (μTongue [microfluidics-on-a-tongue]). (B) *In vivo* imaging preparation for the geniculate ganglion using a microendoscopic gradient refractive index (GRIN) probe. Reproduced from the article of Barretto et al. (2015) (Nature *517*, 373-376) with original copyright holder's permission. (C) Fiber photometry on rostral nucleus of the solitary tract (rNST). Reproduced from the article of Jin et al. (2021) (Cell *184*, 257-271.e16) with original copyright holder's permission. (D) Two-photon imaging of the insular cortex in an awake head-fixed mouse using a prism mirror.

tongue. Physiological fluid from the cochlear promontory may interfere with data acquisition and a tapered suction tip can be introduced to continuously remove the fluid. The acquired temporal traces for each neuron are extracted by manual regions-of-interest or advanced algorithms such as CNMF (Pnevmatikakis et al., 2016) or CaImAn (Giovannucci et al., 2019). To resolve data that are accompanied by excessive noise, deep-learning-based denoising algorithms, such as DeepCAD (Li et al., 2021) or DeepInterpolation (Lecoq et al., 2021), can be used to robustly extract signals of interest.

Calcium imaging of geniculate neurons was used to study the coding logic of peripheral taste information. Barretto et al. (2015) used two-photon microendoscopy in a Thy1-GCaMP mouse to show that geniculate neurons were primarily responsive to a single taste quality. In this study, only a minor portion of the neurons were reported to be responsive to multiple taste qualities, supporting the labeled line coding model. Later, Wu et al. (2015) also performed calcium imaging of geniculate neurons after surgical exposure of the geniculate ganglion. By administering different concentrations of tastants to a mouse, this study revealed that the proportion of neurons responding to multiple taste qualities exhibited a sharp dependence on the concentration of tastants (Wu et al., 2015). Even single-tuned geniculate neurons at low tastant concentrations showed heterogeneous responses to multiple taste qualities at high concentrations. This study suggests that the strict labeled-line coding model may not fully account for peripheral taste encoding (Roper and Chaudhari, 2017).

BRAIN

To measure the activity of neurons in the rostral NST (rNST), where the primary central neuronal station of gustatory information is occupied, electrophysiological recording using a microelectrode is the gold standard approach (Peng et al., 2015). Alternatively, fiber photometry can be used to monitor the calcium activity of genetically targeted neuronal subpopulations in the rNST (Gunaydin et al., 2014; Jin et al., 2021; Zhang et al., 2019) (Fig. 2C). Typically, the time-series acquisition of a fluorescent signal through an implanted multimode fiber is stable even under awake behaving conditions. Fiber photometry was used to reveal that *Pdyn* neurons (sour-sensing neurons) in the rNST respond specifically to sour tastants (Zhang et al., 2019) and that licking behavior activates *Sst*- and *Calb2*-neurons in the rNST (Jin et al., 2021). Furthermore, fiber implants can also be used to deliver optogenetic photostimulation on specific neurons expressing light-sensitive opsins in the rNST (e.g., channelrhodopsin-2) (Jin et al., 2021; Zhang et al., 2019). For example, Jin et al. (2021) showed that optogenetic activation of sour neurons in the rNST induced prototypical acid-aversion behavior.

Although fiber photometry readily provides information on population-level neural dynamics, it lacks the spatial information to discern individual neuronal activities. To overcome this limitation, miniature integrated microscopes (miniscopes) were developed by miniaturizing conventional widefield fluorescence microscopy into a head-mountable size (Ghosh et al., 2011). In the miniscope, a thin GRIN lens (diameter = 0.3-2 mm) replaces the conventional objective lens so that it can be inserted into the targeted brain area with minimal invasiveness. Other parts, including a tube lens, fluorescence filters, a light source, and a camera, are compacted to a small form factor, so they can be mounted on the head of an awake behaving mouse. Although not widely adopted for taste research, miniscopes will be useful in investigating individual neuronal activities in deep brain areas such as the rNST, PbN (parabrachial nucleus), and VPM (ventral posteromedial nucleus) (Fig. 1).

Two-photon fluorescence microscopy is advantageous for observing individual neuronal activities in the insular cortex, which is the primary sensory cortex for gustation. Chen et al. (2011) prepared an open cranial window model at the insular cortex located ~1 mm above the intersection of the middle cerebral artery and rhinal vein. Observing the neuronal response to five basic tastes in an anesthetized mouse, this study revealed the topographic segregation of different taste qualities in the primary gustatory cortex. Another group later introduced a miniature prism mirror and GRIN probe to a two-photon microscope to perform a comparable study in a head-fixed awake mouse (Fig. 2D) (Chen et al., 2021). Notably, the present study reported a scattered distribution of neurons responding to distinct taste qualities rather than topographic segregation.

CONCLUDING REMARK

Optical tools are essential in understanding how taste information is processed from the tongue to the brain. Considering the recent phenomenal advances in optical tools for neuroscience, we anticipate that optical approaches will be increasingly used in taste research. In terms of optical hardware, volumetric imaging techniques, such as light-sheet microscopy (Stelzer et al., 2021), will provide a more comprehensive dataset on each taste organ, for example, on how a taste bud processes taste information from the apical microvilli to the afferent nerves. Adopting advanced genetically encoded indicators will provide access to veiled information, such as membrane potentials (e.g., QuasArs, Archon) or neurotransmitter dynamics (e.g., GRAB-ATP, iGABASnFR), within the taste bud *in vivo* (Bando et al., 2019; Wang et al., 2018). Moreover, computational analysis and modeling advances are necessary to translate the acquired large-scale datasets into scientific knowledge. Inevitably, the taste field is expected to involve interdisciplinary collaborations with optics, genetics, and computational neuroscience.

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AUTHOR CONTRIBUTIONS

G.Y.P. and H.H. wrote the manuscript. M.C. supervised the

paper and provided feedback.

CONFLICT OF INTEREST

The authors have no potential conflicts of interest to disclose.

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