Supplementary Information

Intravital Microscopic Interrogation of Peripheral Taste Sensation

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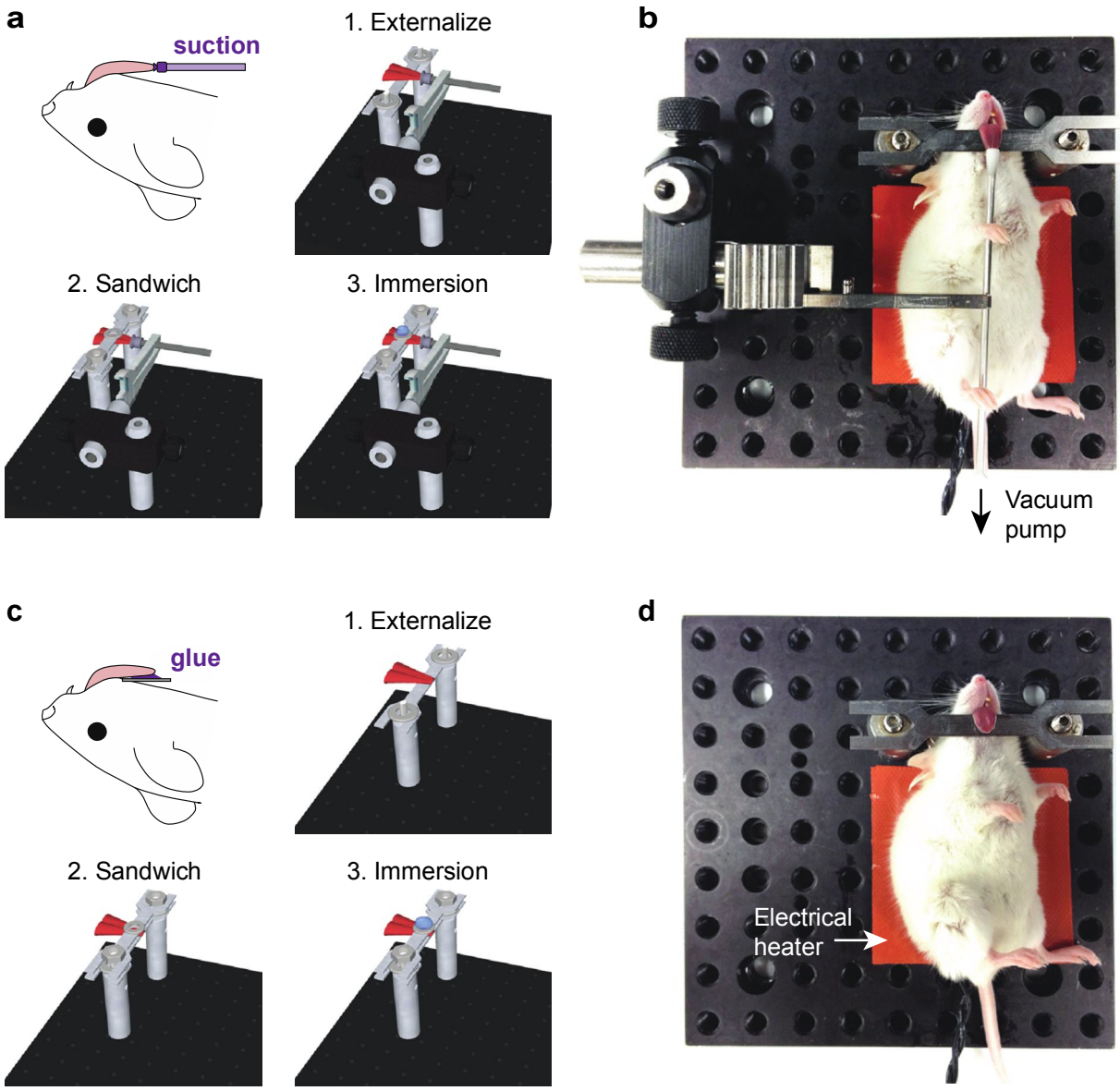


Figure S1. Procedures for Mounting the Tongue on the Holder.

(A) The tongue of an anesthetized mouse is pulled out by using a suction grabber (1.5 mm in diameter), placed onto a metal plate, covered with a top plate with a central hole, and then immersed with artificial saliva (blue).

(B) Photograph before placing the top plate.

(C) Alternative procedures based on gluing the ventral surface of the tongue to the bottom metal plate. The rest of the steps are equivalent.

(D) Photograph of a mouse after the tongue is attached to the plate.

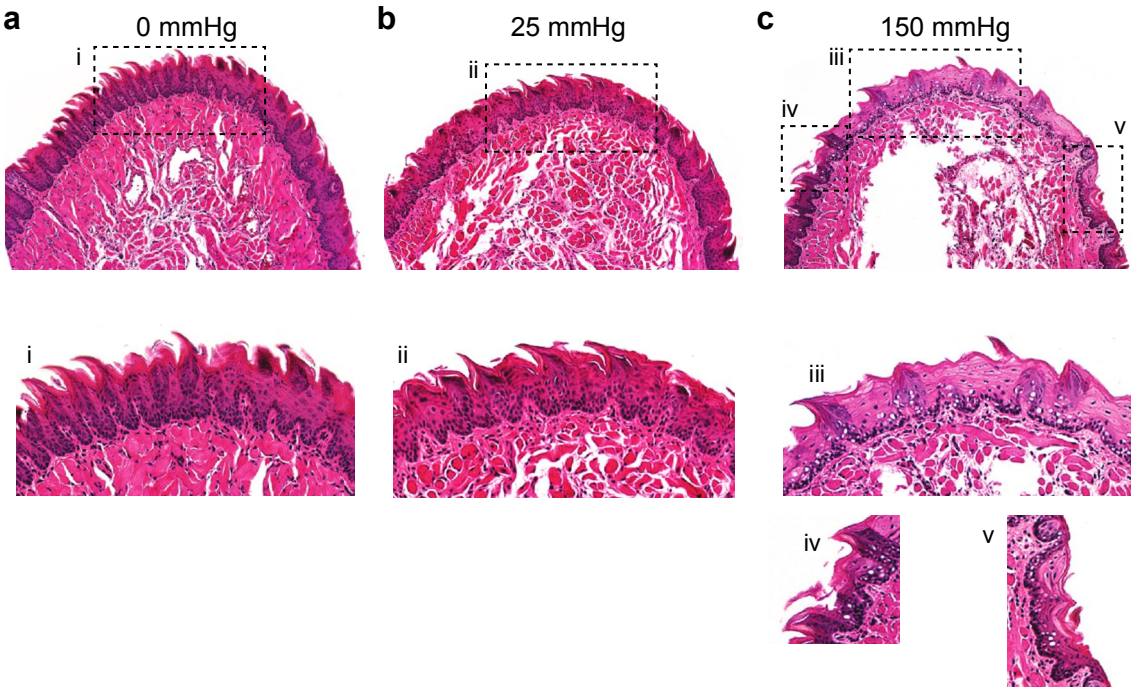


Figure S2. Histological Analysis of Tissue Injury.

(A-C) In several anesthetized animals, different magnitudes of suction pressure — (A) 0 mmHg, (B) 25 mmHg, or (C) 150 mmHg — were applied to the tip of the tongue for 30 min. The tongue was excised, fixed, frozen sectioned, and stained with hematoxylin and eosin (H&E). Bright-field images were acquired with a 4X objective lens. Images in the bottom row are magnified views in the tip of the tongue (dashed rectangle). Arrows indicate tissue deformation by contact to a suction holder. No tissue damages were observed for the modest pressure level of 25 mmHg as in the case of 0 mmHg. Significant tissue damages occurred at the high pressure of 150 mmHg, as expected.

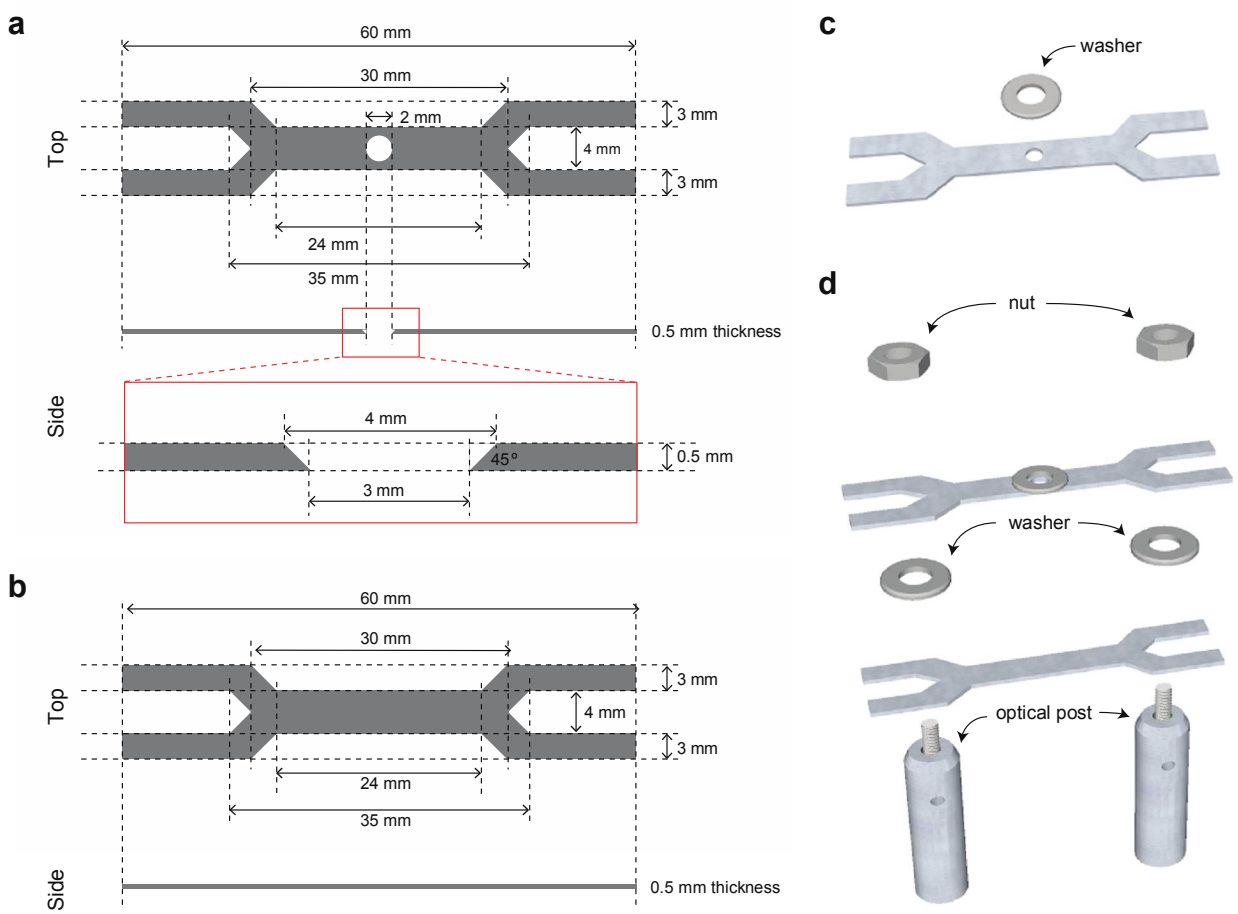


Figure S3. Design of the Tongue Holder.

(A) Drawing of the top metal plate with a central hole.

(B) Drawing of the bottom plate.

(C) A washer was glued to the top plate around the hole to form a round wall to contain immersion solution.

(D) Assembly of the metal pieces.

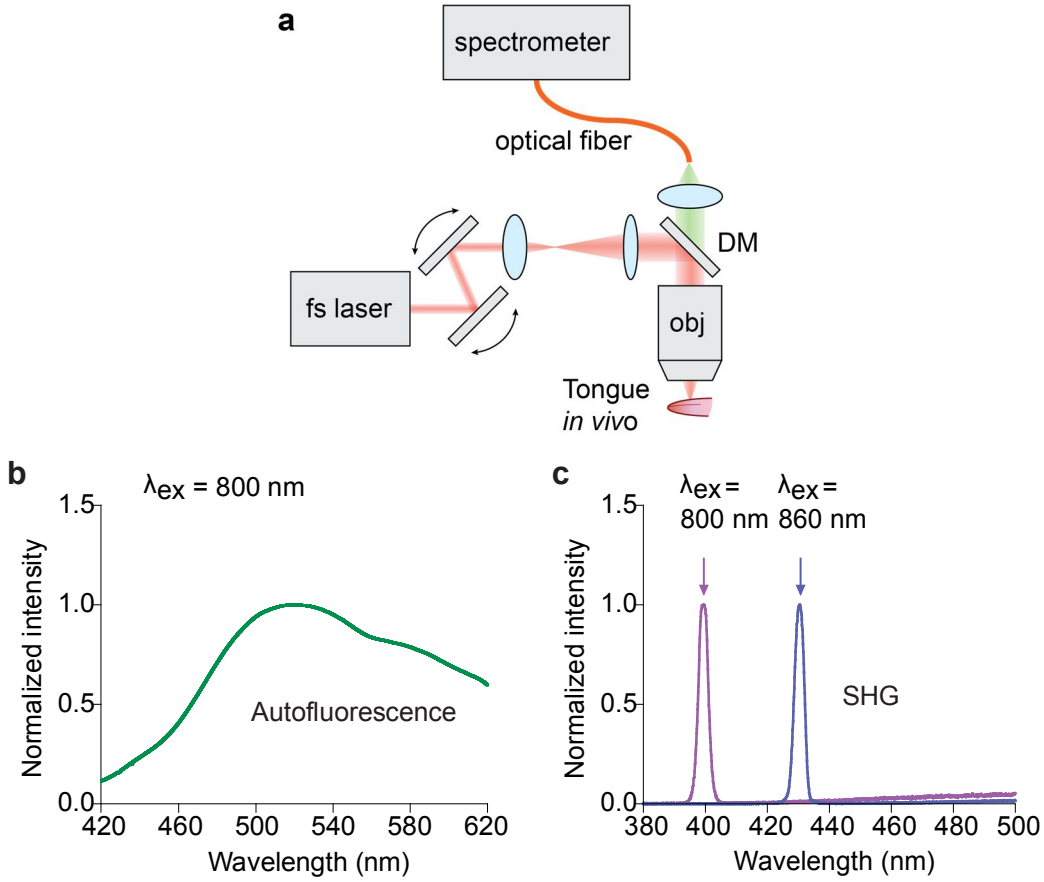
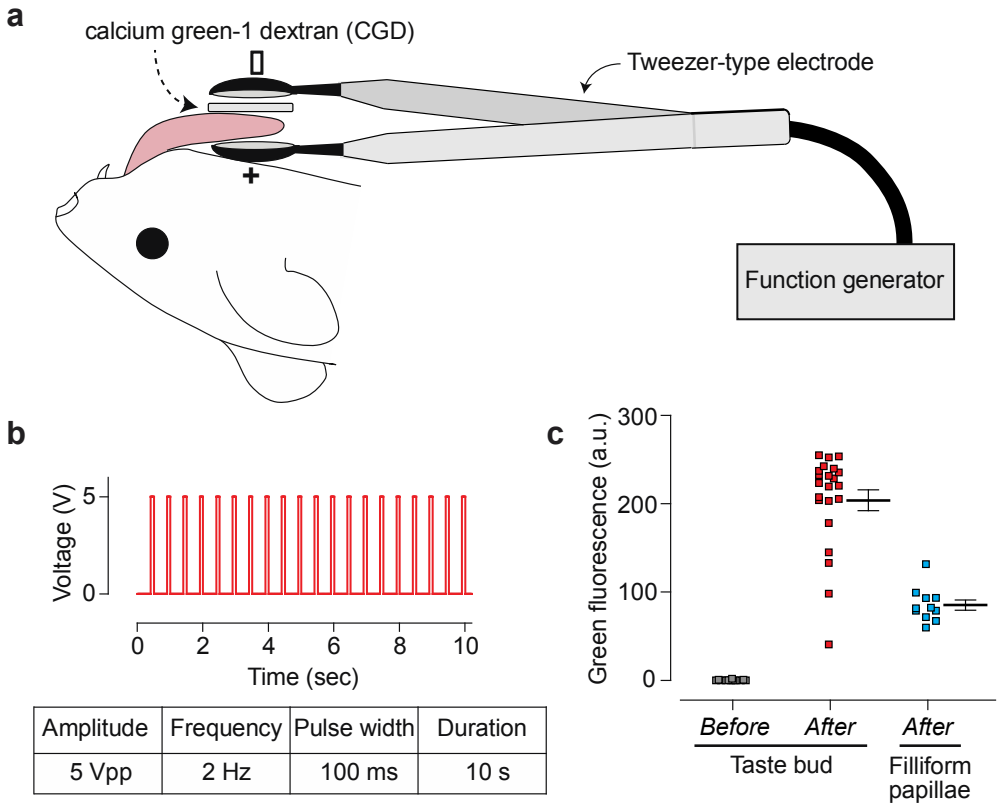


Figure S4. Characterization of Two-Photon Induced Luminescence from the Dorsal Surface of the Tongue.

(A) Optical setup. Photoluminescence from a two-photon microscope was coupled to a two-stage grating spectrometer via a multi-mode optical fiber. DM, dichroic mirror.

(B) The spectrum of two-photon autofluorescence from the keratinized epithelium of the filiform papillae.

(C) Second harmonic generation (SHG) signal from the collagen-rich connective tissue under and around a taste bud. The SHG wavelength was exactly the half of the excitation laser wavelength.



Amplitude	Frequency	Pulse width	Duration
5 Vpp	2 Hz	100 ms	10 s

Figure S5. Electrophoretic Bulk Loading of a Calcium Indicator Dye into Taste Receptor Cells.

(A) Schematic of the setup. A paper tissue soaked with calcium green-1 dextran (CGD) was placed on the dorsal surface of the tongue. electrical pulses were applied across the tongue by a pair of tweezer-type electrodes.

(B) The electrical pulse train used for electroporation.

(C) The magnitude of baseline green fluorescence (500-550 nm) from taste buds before (control) and after (treated) the electrophoresis treatment. Fluorescence in filiform papillae (treated) is substantially weaker, indicating that the calcium dye went into primarily the receptor cells, presumably through the taste pore, but much less to other epithelial cells.

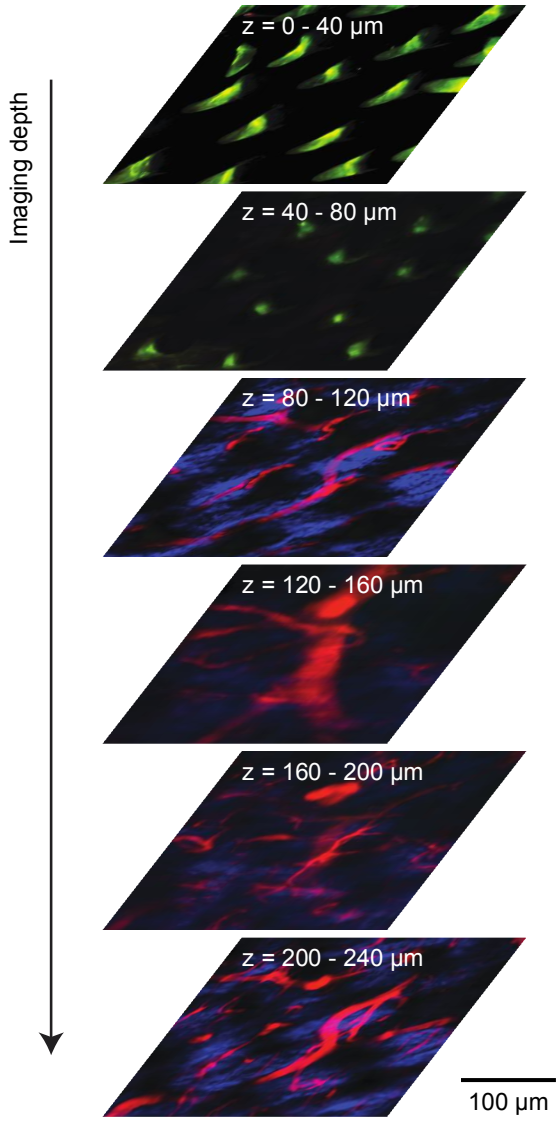


Figure S6. Depth-Resolved Images of the Mucosa.

Each image represents an average of 20 z-stack images taken with 2 µm interval over the depth range indicated. Yellow-green fluorescence is due to autofluorescence primarily from keratin; blue color is the second harmonic signal from collagen fibers; and red fluorescence was generated from intravascular rhodamine dextran.

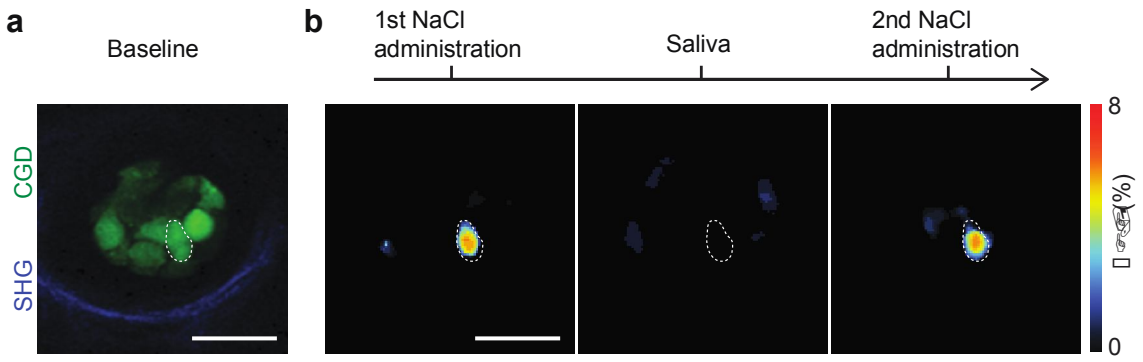


Figure S7. Intraoral Taste Sensation.

(A) Image of a test bud. Green, calcium green-1 dextran (CGD); blue, second harmonic generation (SHG) signal.

(B) Calcium indicator response to repeated stimulus with salty tastant, 180 mM NaCl in artificial saliva, applied to the apical side of the tongue. Artificial saliva was used in between the salty stimuli.

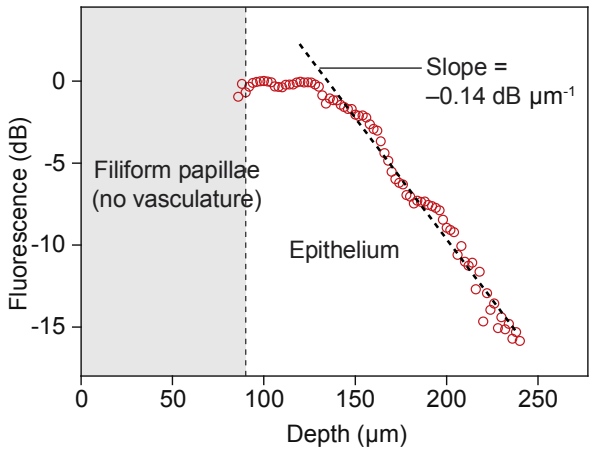


Figure S8. Depth-Dependent Fluorescence Intensity in the Tongue Mucosa.

Red circles, fluorescence intensity of intravascular rhodamine dextran measured as a function of depth; Dotted line, linear fit (R^2 = 0.993) in the depth range of 120 to 240 μm.

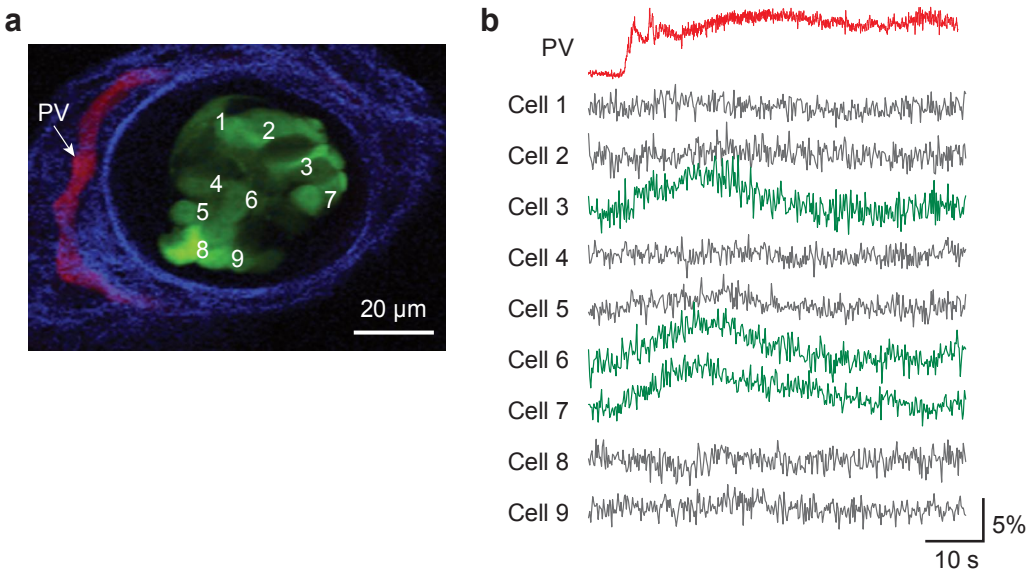


Figure S9. Intravascular Taste Sensation.

(A) Image of a taste bud. Green, fluorescence from calcium-green dextran in receptor cells; Blue, SHG from collagen fibers; Red, red fluorescence from pericellular blood vessel (PV).

(B) Top, fluorescence indicating the arrival of tastant (Na-saccharine) in the PV. Below, Calcium traces of the receptor cells in (A). Cells 3, 6, and 7 were activated, responding to intravenous Na-saccharine.

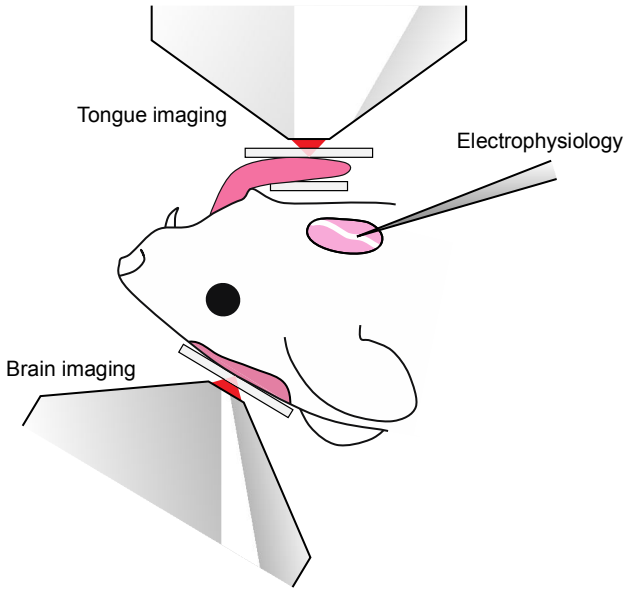


Figure S10. A Scheme for Comprehensive Study on Taste Transduction Pathway.

A possible configuration for imaging of the tongue and brain, as well as nerve electrophysiology for the systems study of taste transduction pathways is illustrated.

Supplementary Video S1. Three-dimensional Microanatomy of a Taste Bud In Vivo

This video shows 3D rendered view of a taste bud in the anterior tongue of a live mouse. Z-stacked images were acquired using a two-photon microscopy with 2-μm axial spacing and rendered with an open software ZEN (Zeiss).

Supplementary Video S2. Microvascular Perfusion in a Taste Bud

This video shows blood flow around a fungiform papilla in a live mouse taken with a video-rate two-photon microscopy at a frame rate of 30 Hz. The blood plasma is stained with Rhodamine-dextran (red). TB, taste bud.