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Isolating Taste Buds and Taste Cells from Vallate Papillae of C57BL/6J Mice for Detecting Transmitter Secretion

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[Abstract] Mouse is a well-accepted model for studying taste bud function. Mice readily detect and respond to taste substances that humans consider to have sweet, bitter, salty, sour and umami taste qualities. A great deal of recent research on taste receptors is based on this species. Live mice are needed for these experiments because no alternative *in vitro* model incorporates all elements of taste transduction and peripheral signaling. The C57BL/6J strain was selected because these mice respond robustly to many taste stimuli and because of variety of transgenic animals, such as PLCβ2-GFP and GAD67-GFP, were derived from that strain. Prior analyses on behavior, nerve responses, cellular electrophysiology and molecular biology, all conducted on C57BL/6J mice will form a solid foundation for the proposed studies (Finger *et al.*, 2005; Huang and Wu, 2015; Huang *et al.*, 2007). Thus, freshly euthanized animals must be used as a source of taste buds from which we will isolate taste buds and taste cells.

Materials and Reagents

1. Fire-polished borosilicate glass micropipette (World Precision Instrument) with suction apparatus (Figure 1D)
   a. Polyethylene tubing (BD, Intramedic™, model: PE#205)
   b. 1 ml syringe (BD)

   Caution: Pipette tip must be large enough to allow taste buds to easily pass through the opening (60~80 µm and 20~30 µm for taste buds and taste cells collection, respectively). However, a wide opening will result in a large volume of solution being drawn into the suction pipette.
2. Sylgard dish with dissecting pins (Figure 2A)
3. Eppendorf centrifuge tubes (1.5 ml)
4. 35 mm culture dishes (Corning)
5. Plastic two-way valve of syringes (Figure 1D)
6. Cell-TAK cell and tissue adhesive (Corning) coated coverslip(s)

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Caution: Apply a tiny drop of Cell-TAK onto the center of 12-mm coverslips. Wait until the droplet is completely dry. Use ethanol (75%) followed by nanopure UV water to rinse and clean coverslips.

7. C57BL/6J strain mice (the Jackson laboratory) (Figure 1A)
8. 100% CO_2
9. Nanopure UV water (Thermo Fisher Scientific)
10. Collagenase A (Roche Diagnostics, catalog number: 10103578001)
11. Dispase II (Roche Diagnostics, catalog number: 04942078001)
12. Elastase (Worthington Biochemical Corporation, catalog number: 2292)
13. Trypsin inhibitor (Roche Diagnostics, catalog number: 10109886001)
14. Calcium chloride ($CaCl_2$) (Sigma-Aldrich, catalog number: C5670)
15. HEPES (Sigma-Aldrich, catalog number: H4034)
16. Potassium chloride (KCl) (Sigma-Aldrich, catalog number: P5405)
17. Magnesium chloride ($MgCl_2$) (Sigma-Aldrich, catalog number: M4880)
18. Sodium chloride (NaCl) (Sigma-Aldrich, catalog number: S7653)
19. Sodium bicarbonate ($NaHCO_3$) (Sigma-Aldrich, catalog number: S6297)
20. Sodium pyruvate (Sigma-Aldrich, catalog number: P5280)
21. D-(+)-Glucose (Sigma-Aldrich, catalog number: G7021)
22. BAPTA (EMD Millipore Corporation, Calbiochem, catalog number: 196418)
23. Ethylene glycol-bis(2-aminoethylether)-N, N, N', N'-tetraacetic acid (EGTA) (Sigma-Aldrich, catalog number: E0396)
24. Enzyme mixture (see Recipes)
25. Tyrode's buffer (see Recipes)
26. Ca^{2+}/Mg^{2+}-free Tyrode's buffer (see Recipes)

Equipment

1. Olympus IX73 inverted fluorescence microscope (Olympus Imaging America Inc., Olympus Optical)
2. Stereo dissecting microscope (ZEISS, ZEISS Optical) (Figure 1C)
3. Fiber optical illuminator (Dolan-Jenner Industrial Inc.)
4. Compressed CO_2 gas in the cylinder (Figure 1B)
5. Plastic chamber connecting with an ample length of vinyl tubing
6. XSE analytical balance (Mettler-Toledo International Inc.)
7. Single-sample micro osmometer (Advanced Instruments, model: 3320)
8. Thermo Scientific benchtop pH meter (Thermo Fisher Scientific, Accumet™, model: XL15)
9. Narishige microforge for fire polishing with tungsten filament, electrically heated (NARISHIGE Group, model: MF-900)

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10. Flaming/Brown glass micropipette puller (Sutter Instruments, model: P-1000)
11. Recording/Perfusion chamber (Warner Instruments, model: RC-25) (Figure 2B)
12. Magnetic platform (PH4) for RC-25 recording chamber, and for mounting onto an Olympus IX73 microscope (Figure 2B)
13. Thermolyne stirrer (Thomas Scientific, model: Nuova II)
14. Spectrafuge 7M microcentrifuge (ReGen Lab Equipment, model: SKU: W095074)
15. Eppendorf 5702 centrifuge (Thermo Fisher Scientific, Fisher Scientific™, model: Eppendorf 5702)
16. VWR vortex mixer for vortexing the enzyme mix (VWR International, model: MINI 230V)
17. Gilson pipettors (200 µl and 1 ml tips) (VWR International)
18. Sharp tweezers
19. Microsurgical scissors

Procedure

1. Mice are euthanized by cervical dislocation under deep CO_2 (100%) anesthesia (Figure 1A-B).
2. Obtain the tongue from animals and place in Tyrode's buffer.
3. Pin tongue on a Sylgard dish so that vallate papillae are easily accessible (Figure 1C).
4. Inject ~0.3 ml of enzyme mixture in the regions near the vallate papillae (on each side) subepithelially. The needle should be visibly beneath the epithelium.

   Note: Delivering enzyme mixture by inserting the needle too deep will result in the digestion of the muscle tissue. If the location of needle is too shallow, the epithelium will be pierced, the enzyme mixture may leak through the holes on the lingual epithelium.

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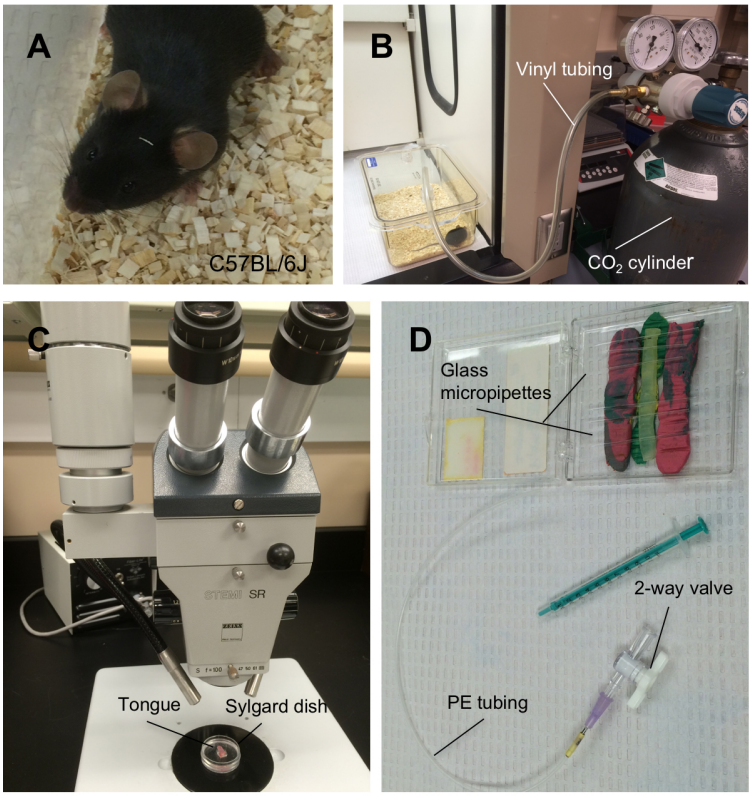

Figure 1. Euthanization of mice, and excision of tongues. The photograph shows (A) the animal, C57BL/6J mouse, (B) the apparatus for euthanizing animals, (C) the tongue pinned on a Sylgard dish under the stereo dissecting microscope, and (D) the suction apparatus for collecting taste buds.

5. Incubate the tongue in 5~10 ml of Tyrode's buffer with aeration for 30 min.
6. Re-pin the tongue so that vallate papillae are readily accessible (Figure 1C).
7. Cut the epithelium surrounding vallate papillae with microsurgical scissors.
8. Peel the epithelium away from the tongue using a sharp set of tweezers.
9. Pin the pieces of epithelium, serous side up, onto a Sylgard dish. Stretch the epithelium so that each papilla is exposed and easily accessible (Figure 2A).
10. Exchange the solution for Ca^{2+}/Mg^{2+}-free Tyrode's buffer. Incubate the epithelium in the buffer for 20 min for collecting individual taste buds or 30 min for single taste cells.
11. Replace Ca^{2+}/Mg^{2+}-free Tyrode's buffer in the Sylgard dish with regular Tyrode's buffer.
12. Using a fire-polished glass micropipette (60~80 μm) (Figure 1D), sweep each papilla several times while applying suction. Periodically stop and discharge the solution onto a Cell-TAK treated coverslip.

    Critical: There are, typically, sufficient number of taste buds on the vallate papillae to make 2~4 coverslips.

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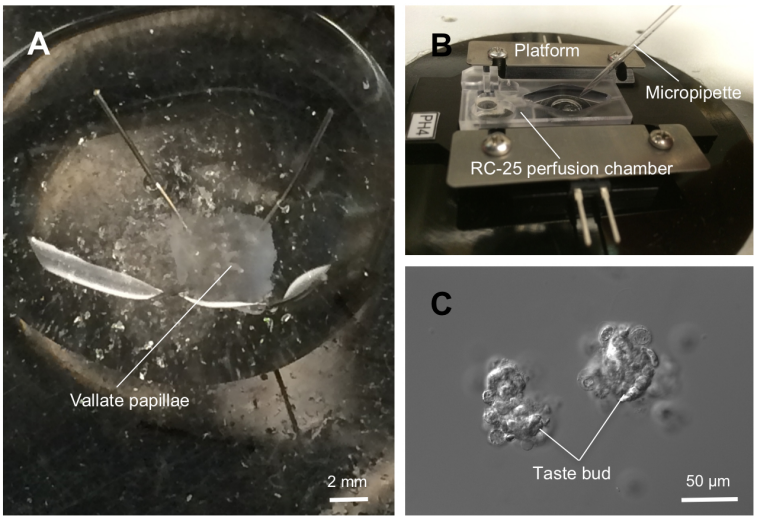

Figure 2. Suction apparatus setup to collect taste buds from mouse vallate papillae. A. The photo shows the peeled lingual epithelium, serous side up, pinned on a Sylgard dish. B. Isolation of taste buds is performed in the Sylgard dish, and then taste buds are delivered via a glass micropipette to a recording chamber assembled on the platform. C. A Nomarski optics micrographic image of isolated taste buds in a living preparation when viewed under the Olympus IX73 microscope.

13. For collecting single cells, gentle trituration (suck Tyrode's containing taste buds into the glass pipette and discharge it immediately for repeating 5-6 times) is required via 20~30 μm fire-polished glass pipettes.
    Note: Validating the intact membranes can be a criterion for healthy-looking taste buds/cells. Taste cells may be not healthy if the intracellular Ca^{2+} concentration is over 200 nM at their resting stage (Critical!).
14. Allow 20 min for taste buds and/or taste cells to settle, and to attach to the bottom of the chamber, which is made by glass coverslips (Figure 2B-C).

Recipes

1. Enzyme mixture

Collagenase A	1 mg/ml
Dispase II	2.5 mg/ml
Elastase	1 mg/ml
Trypsin inhibitor	1 mg/ml

2. Tyrode's buffer
   310-320 Osm and applied at pH 7.2

$CaCl_2$	2 mM
HEPES	10 mM

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KCl	5 mM
$MgCl_2$	1 mM
NaCl	140 mM
$NaHCO_3$	10 mM
Na-pyruvate	10 mM
Glucose	10 mM

3. Ca^{2+}/Mg^{2+}-free tyrode's buffer (pH 7.2)

$CaCl_2$	0 mM
HEPES	10 mM
KCl	5 mM
$MgCl_2$	0 mM
NaCl	140 mM
$NaHCO_3$	10 mM
Na-pyruvate	10 mM
Glucose	10 mM
BAPTA	2 mM
EGTA	2 mM

Acknowledgments

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