Taste 1: Periphery

Anatomy of Tongue and Gustatory Nerves

Taste Transduction Mechanisms
    Ion channels (Na+, H+)
    G-protein coupled receptors
    (sweet, bitter, umami)

Second Messengers
    gustducin, cAMP, Ca++

Transmission to the Nerve

Gustatory Nerve Responses

Principles of Gustatory System

Detection of water-soluble chemicals in mouth or on substrate

Labeled line with intensity coding (not a complex neural code, little peripheral processing)

Tightly coupled to physiological and motivation systems

Taste receptors evolved as means to retain information about ingestive environment across generations.

Learning as means to retain information about ingestive environment across an animal's lifetime.

Taste Receptive Mechanism

soluble ligand
receptors in mouth (or feet, barbells, etc.)
stimulate gustatory nerves (CN VII, IX, X)
not mediated by somatosensation (V) or olfaction (I)
drives ingestive behavior & physiology

Do we define taste by ligand?

sucrose (mono-/di-saccharides)

by receptor?

T1R2/T1R3 (sweet receptor)

- *orphan receptors with unknown ligand*
- *ligands with unknown receptors*
- *receptors found in rest of body*

by behavioral response?

drives ingestion in all species

by subjective sensation?

sweet!

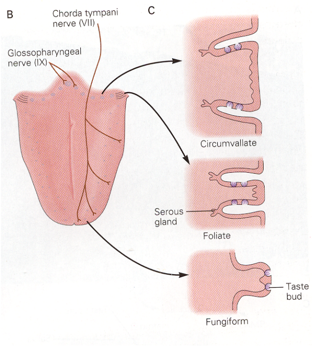
Taste - Regional organization

Anterior 2/3 innervated by geniculate ganglion, (Chorda tympani, VII)

Fungiform papillae (sweet, salt)
- peg-like structure
- 1-5 taste buds

Posterior 1/3 innervated by petrosal ganglion (Glossoppharyngeal, IX)
- (bitter)
- Foliate papillae
- Circumvallate papillae

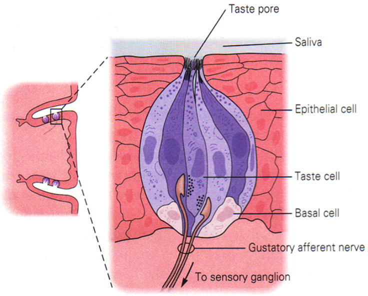
In rat:
soft palate innervated by superficial petrosal nerve (sweet)



Methylene Blue staining of taste papillae

Colorado State ETextbook

Taste Papilla Anatomy



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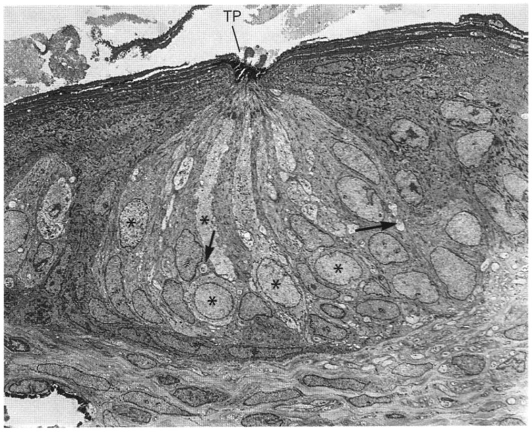
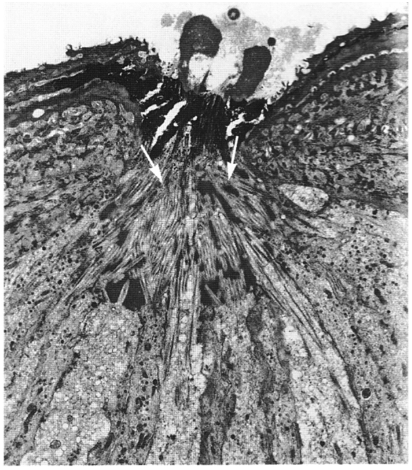
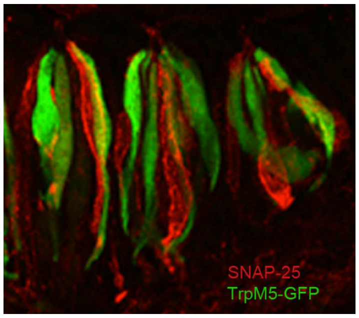
and taste pores



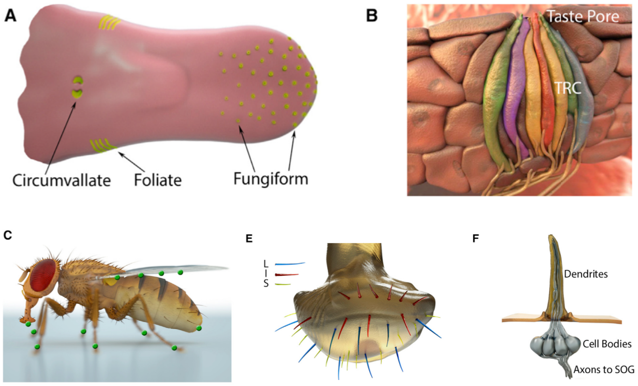


Taste Buds



Type I glial-like cells
    salt?
Type II Receptor cells
    sweet, umami, bitter
Type III Presynaptic Cells
    sour

Taste buds consist of 50 to 100 taste cells that are roughly 10 μm across and about 100 μm in height. Taste stimuli contact the apical (top) tips of the cells, while afferent nerve fibers contact the basolateral (lower) portions of the taste cells.



Liman, Neuron 81 (2014) 985

Taste Qualities

sweet (mono/di-saccharides)
umami (glutamate & other amino acids)
bitter (none of the above)

salt (sodium & other alkali cations)
sour (acids)

fats/fatty acids
polycose in rodents (polysaccharides)
(other taste qualities we are not aware of?)

Not Taste

Flavors (e.g. olfaction)
Texture (e.g. creaminess)
Stringency
Temperature (e.g. spicy capsaicin)

although multimodal sensation clearly integrated (in cortex?) to make a synthetic flavor/mouth feel

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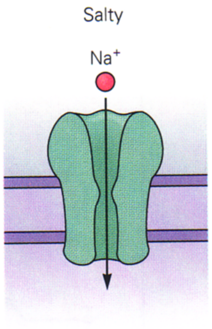
Taste	Substance	Threshold for tasting
Salty	NaCl	0.01 M
Sour	HCl	0.0009 M
Sweet	Sucrose	0.01 M
Bitter	Quinine	0.000008 M
Umami	Glutamate	0.0007 M

Colorado State ETextbook

Epithelial Sodium Channel (ENaC)



allows Na, Li, K to enter cell -> depolarization

Present along lining of GI tract et al. (mouth, intestine, lung, kidney)

Blocked by amiloride

upregulated by aldosterone (i.e. when sodium depleted)

additional channels contribute to salt taste, esp. in humans

Sour Taste through H+ channels & pH sensors

Hydrochloric acid, citric acid, ammonia

H+ passes through ENaC or K+ channel, TRP channels, etc.

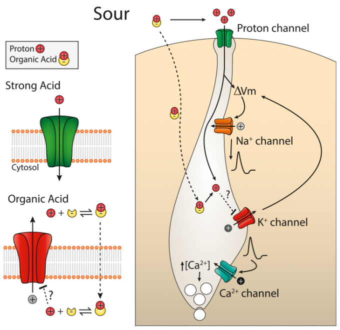
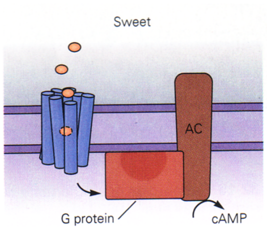


Figure 3. Sour Taste
Sour taste in vertebrates is initiated when protons enter through an apically located proton-selective ion channel. Weak acids may also activate sour cells by penetrating the cell membrane and acidifying the cytosol, leading to closure of resting K^+ channels and membrane depolarization.

Liman, Neuron 81 (2014) 985

Sweet Taste



Sucrose - prototypical sweet taste (all animals love sweet)

some amino acids (phenylalanine)

Artificial sweetners:
- saccharin (lo conc)
- cyclamate
- aspartame

(detection of sweeteners is species specific)

Sweet & Umami Taste Receptors

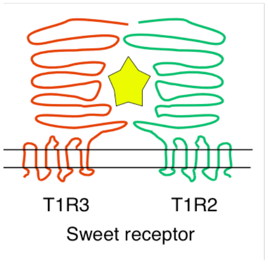
T1Rs
not co-expressed with gustducin
function as heterodimers
elevate Ca++

T1R3
mapped to Sac locus in mice
expressed in many cells across tongue

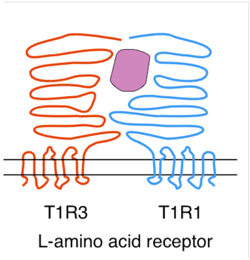
T1R1
expressed on front of tongue and palate

T1R2
expressed on back of tongue

T1Rs are expressed as heterodimers

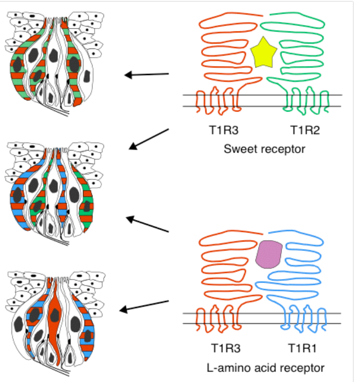


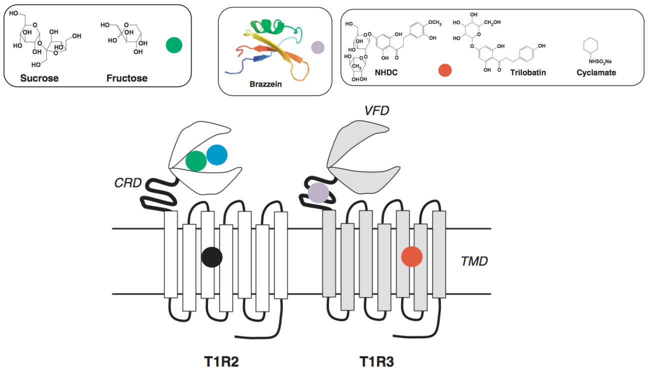
(Sucrose)



(Umami)

Different taste cells & buds express different complements of T1Rs

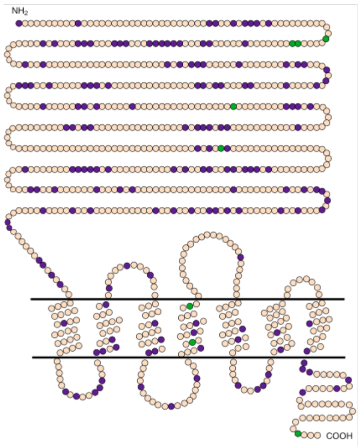




Structure of the sweet taste receptor and depiction of representative modulators. The sweet receptor is composed of two different subunits named T1R2 and T1R3 which in turn contain three distinct domains, the venus flytrap domain (VFD), the cysteine-rich domain (CRD) and the heptahelical transmembrane domain (TMD). Agonists and positive allosteric modulators interact on these different domains of the heterodimer, as depicted by the colored dots within the receptor subunits.

Servant Trends.Pharmacol Sci. 32(2011)631

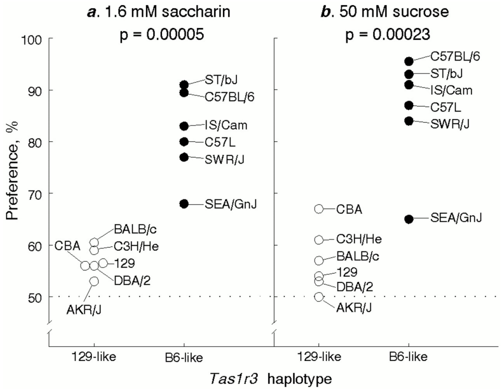
T1R3 sweet receptor



Green = Sac polymorphisms

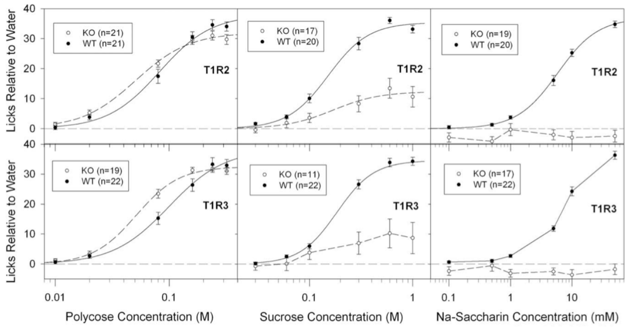
Blue = T1R common a.a.

Sac Locus and Sweet Preference in Mouse Strains



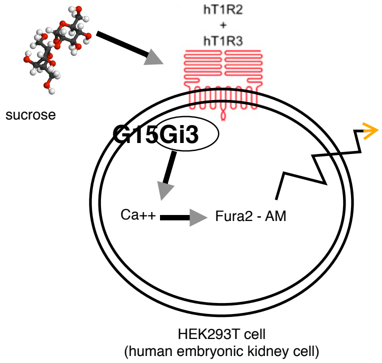
Bachmanov Chem Sen 2001

T1R2 or T1R3 KO reduces behavioral response to sweeteners



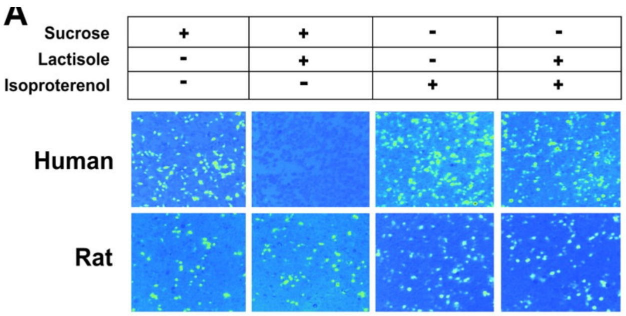
Spector and Glendinning, Current Opinion in Neurobiology 2009, 19:370–377

In this assay system, the human sweet taste receptor couples to the promiscuous G protein Gα15 to induce PLC activation, causing a net increase in calcium mobilization inside cells.



based on Servant, PNAS 107 (2010) 4746

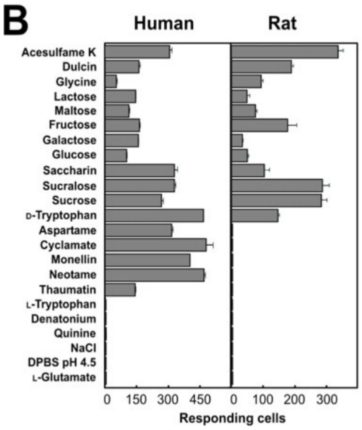
Calcium response of cells transfected with human or rat T1R2 and T1R3



Sucrose	+	+	-	-
Lactisole	-	+	-	+
Isoproterenol	-	-	+	+

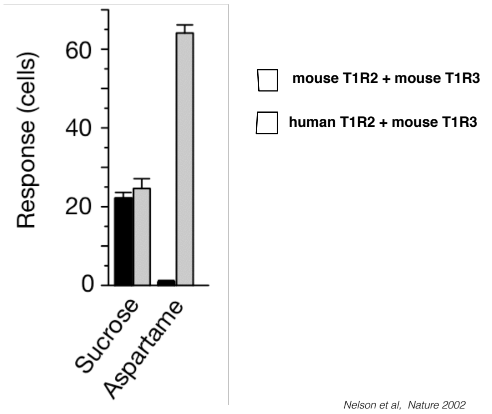
lactisole = sweet inhibitor for humans
isoproterenol = beta-adrenergic agonist (positive control)

Li et al, PNAS 2002



Li et al, PNAS 2002

Mixing T1Rs to explain species differences

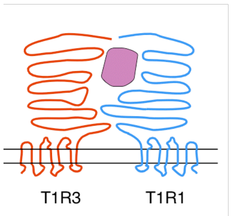


Nelson et al, Nature 2002

Umami Taste and Glutamate Receptors

L-Amino acids detected by:
heterodimers of T1R3 & T1R1

Also, taste-specific splicing of mGluR4; other neuronal GluRs found in taste cells as well



L-amino acid receptor

Bitter Taste

Quinine - prototypical bitter taste
alkaloids - common bitter poisons
many other compounds are bitter…
some species tolerate bitter tastes

T2Rs

- Mediate bitter taste
- ~20-40 different genes
- SOA locus in mice (cluster of 25 T2Rs)
- PROP locus in humans (chromosome 5)
- expressed in back of tongue
- co-expressed with gustducin (G-protein)
- also in gut

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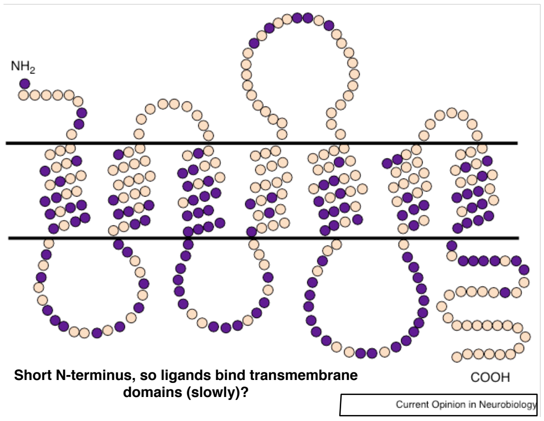
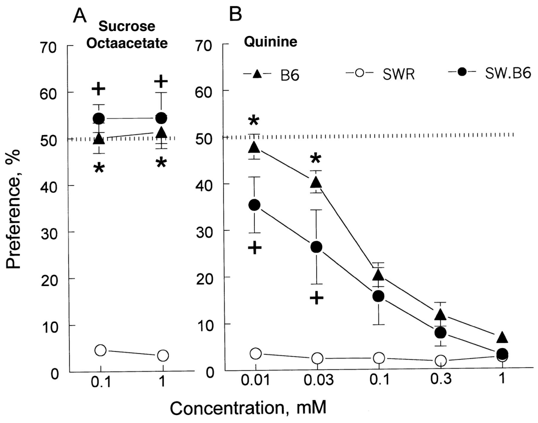
T2R Bitter receptor



Short N-terminus, so ligands bind transmembrane domains (slowly)?

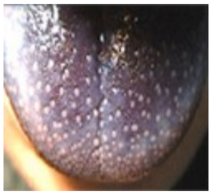
Montmayeur 2002

SOA locus influences bitter taste

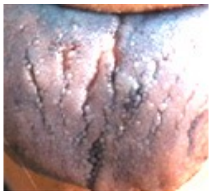


Non-Taster

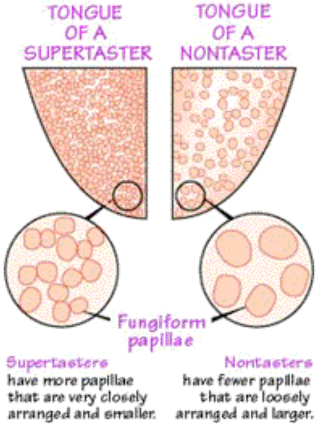
Medium Taster



Taster



Cornell Nutritional Sciences.



PROP sensitivity = polymorphism in bitter receptors

more activity
-> more neurotrophic activity
-> more innervation
-> more taste

Phenylthiocarbamide (PTC)
Propylthiouracil

Scientific American Frontiers, www.pbs.org

TAS2R38 = PROP sensitivity

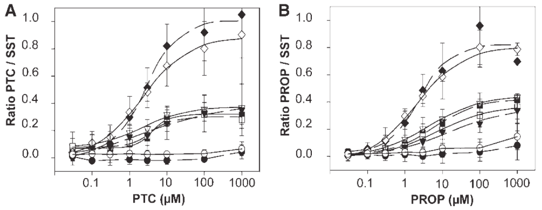
Three most common polymorphisms:

amino acid 49: P or A
amino acid 262: A or V
amino acid 296: V or I

P49, A262-> super taster

A49, V262-> non taster

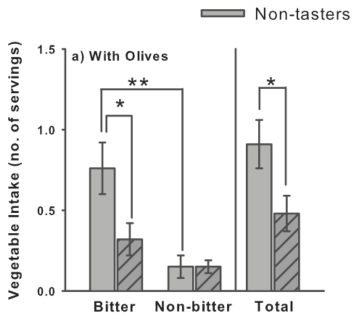
Variants of Tas2R38 expressed in cells: Ca++ response to PROP



C

	receptor variant	EC_{50} PTC [μM]	threshold PTC [μM]	EC_{50} PROP [μM]	threshold PROP [μM]
supertaster->	PAV -◆-	**1.1 ± 0.5**	**0.02 ± 0.02**	**2.1 ± 0.9**	**0.06 ± 0.05**
	PAI —◇—	1.2 ± 0.1	0.12 ± 0.10	1.6 ± 0.3	0.04 ± 0.03
	PVI —▽—	3.1 ± 2.2	0.19 ± 0.18	2.3 ± 0.3	0.03 ± 0.01
	AAI -■-	**2.0 ± 0.3**	**0.35 ± 0.35**	**2.3 ± 0.8**	**0.17 ± 0.04**
	AAV —□—	2.3 ± 1.5	0.05 ± 0.01	2.7 ± 1.6	0.09 ± 0.10
	PVV -▼-	2.1 ± 0.4	0.18 ± 0.07	3.5 ± 1.2	0.06 ± 0.03
	AVV —○—	--	--	--	--
nontaster->	**AVI** -●-	**--**	**--**	**--**	**--**

Bufe, Current Biology, Vol. 15, 322–327, 2005



Mean number of vegetable servings (± SEM) consumed by nontaster and taster preschool children during a free-choice snack test. (a) Vegetables were grouped into bitter (black olives, cucumber, raw broccoli) and nonbitter (red peppers, carrots) categories. **Nontasters consumed more bitter vegetables than nonbitter vegetables.** Additionally, **nontasters consumed more bitter vegetables as well as more vegetables overall than did tasters**.

Tepper. Annu. Rev. Nutr. 2008.28:367-388

Evolution of Bitter Receptors

> Then of course there is the pleasant or unpleasant quality of a sensation ; and this, too, is in general related to our way of life. I will take one example.
>
> Both lead acetate and sugar taste sweet ; the former is a poison, but very rare in nature ; the latter is a useful food, and common in nature. Accordingly we most of us find a sweet taste pleasant.
>
> But if lead acetate were as common in nature as sugar, and sugar as rare as lead acetate, it is safe to prophesy that we should find sweetness a most horrible taste, because we should only survive if we spat out anything which tasted sweet.

Julian Huxley, 1933

Bitter receptors as toxiceptors

sugar/sodium/amino acids

~3 specific receptors to recognize specific essential nutrients

olfaction

1000s receptors required to <u>uniquely</u> distinguish, by comination, all violatile chemicals/hydrocarbons?

immune system

stochastic recombinatorial receptors to each specifically recognize a non-self protein

nocioceptors

various, to recognize all noxious stimuli by presence of cytoplasmic chemicals, extremes of osmolality, pH and temperature

bitter receptors

~40 receptors required to <u>generally</u> detect small soluble chemicals non-nutrient chemicals (potential toxins)?

Summary of Taste Receptors

Taste	Receptor	Agonist	Antagonist
Salt	ENaC	Na, K, Li	amiloride
Sour	ENaC, K+	HCl, NH4Cl	miraculin
Sweet	T1R2/T1R3	sucrose, phenylalanine	gymnemic acid, lactisole
Bitter	T2Rs	quinine, denatonium	?
Umami	T1R1/T1R3, mGluR4	glutamate	?

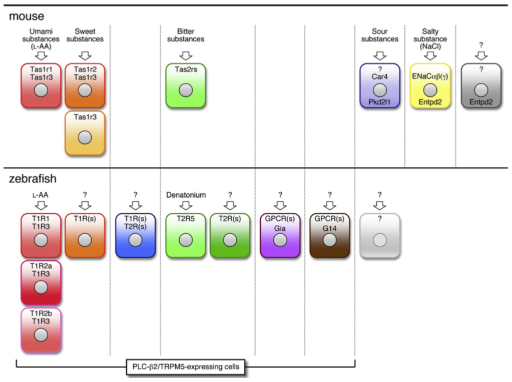


Fig. 1. Diverse array of taste cells in mouse and zebrafish. Types of taste cells are illustrated with specific molecular features such as taste receptors and (in)dispensable markers. Their ligands identified thus far are indicated. Unidentified receptors and ligands are shown by question marks. The unidentified receptors in PLC-β2/TRPM5-expressing taste cells are presumably GPCRs [32]. Pkd2l1 is not a sour receptor channel, because its knockout had little effect to sour response in gustatory neurons [70]. ENaC is indispensable for NaCl attraction [48], but it remains unclear whether it is the specific receptor. Many types of T1R(s)-expressing and T1R(s)/T2R(s)-expressing cells exist in zebrafish, depending on the expression pattern of receptor genes. Taste buds in zebrafish have many cells that do not express PLC-β2 or TRPM5, although little is known for their molecular features.

I. Matsumoto et al. / Seminars in Cell & Developmental Biology 24 (2013) 210–214

Second Messenger Systems

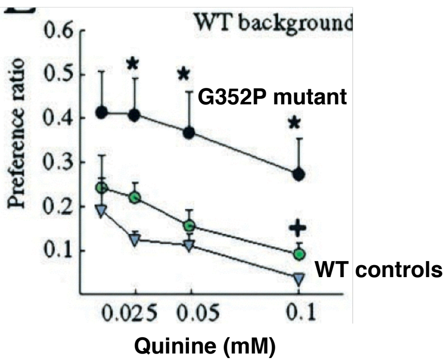
G-Protein coupled receptors

Gustducin
Gi protein similar to transducin in photoreceptors

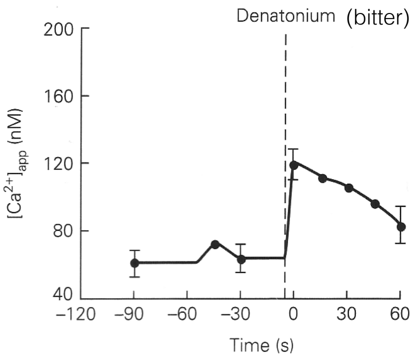
cAMP
decreased for bitter, increased for sweet

Ca++
increased by bitter

Gustducin mutant mice do not taste bitters

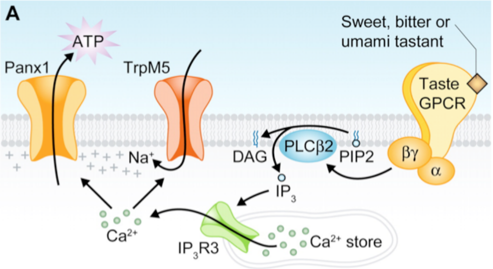


Calcium from internal stores released by bitter



T1, T2 GPCR -> ⬆ internal Ca^{++}

-> ⬆Na^{+} via TrpM5 channel



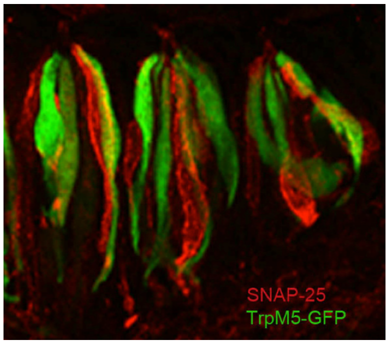
Taste Cell Neurotransmitters

ATP
Serotonin (uptake of 5HT from plasma)
Norepinephrine
Cholecystokinin

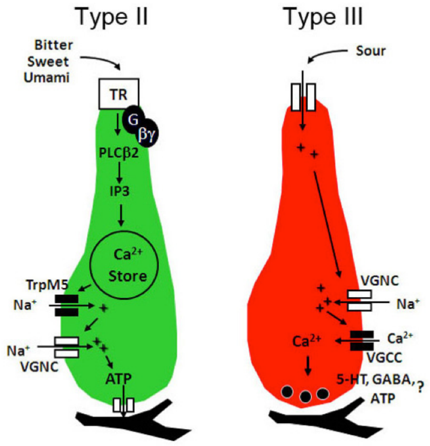
Paracrine Effects:
receptors expressed in adjacent taste cells
transmitters are released from cells that are some distance from nerve.

Modulation by circulating hormones (e.g. leptin)

Taste Cells



Type II Receptor cells: sweet, umami, bitter receptors
lack presynaptic vesicular machinery, but release ATP
Type III Presynaptic Cells: express channels for sour
VGCC-mediated vesicular release of ? (5HT, GABA)



Type III Taste cells release 5HT

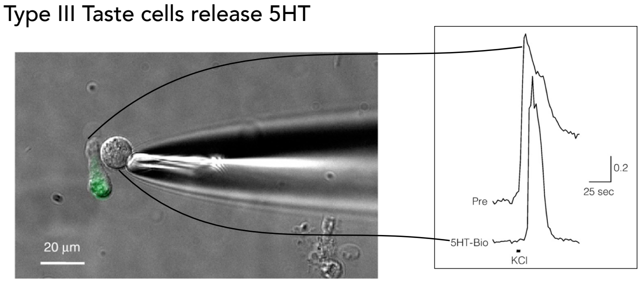


Fig. 2. CHO cells stably expressing high affinity receptors for neurotransmitters constitute effective biosensors for identifying taste transmitters. This micrograph shows a biosensor cell, held on the tip of a glass micropipette and apposed to a Presynaptic Cell isolated from a mouse vallate papilla. Presynaptic taste cells isolated from transgenic mice that express GFP on the GAD67 promoter fluoresce green [24], allowing confident identification of this cell type, as shown here. Presynaptic cells, when depolarized by KCl, secrete serotonin, and in some cases (~33%) co-secrete noradrenalin with serotonin. (No cells were found with depolarization-evoked noradrenalin alone). Modified from [25].

Ca++ imaging in taste cell (Pre) and biosensor cell with 5HT-2C receptors (5HT-Bio)

S.D. Roper / Physiology & Behavior 97 (2009) 604–608

Type II cells release ATP

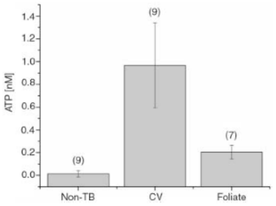
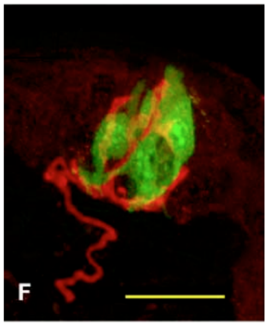


Fig. 4. Release of ATP from taste epithelium when stimulated with a bitter mixture containing denatonium and quinine. Nongustatory epitheli-

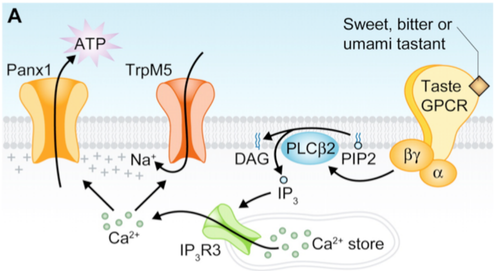
Taste cells are innervated by ATP-receptor bearing fibers

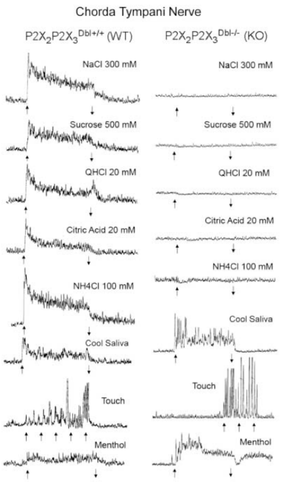


(F) Laryngeal taste buds are innervated by purinoceptive nerve fibers expressing P2X2 (red). Gustducin-expressing taste cells are green. This suggests that laryngeal taste buds, like lingual taste buds, rely on ATP as a transmitter.

Finger, et al. Science 310, 1495 (2005);

Nonvesicular Release of ATP from Type II cells via Panx channels





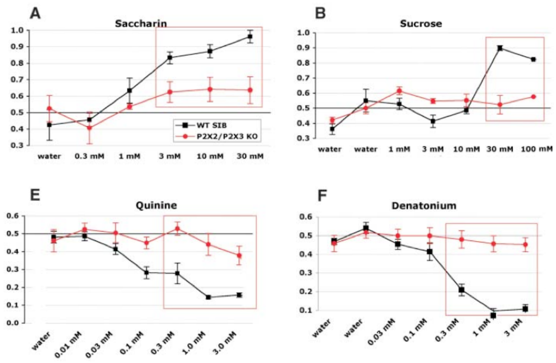
Knockout of ATP receptors (P2X2 & P2X3) abolishes gustatory nerve responses

Gustatory nerve recordings from P2X2 / P2X3Dbl -/- (KO) and P2X2/ P2X3Dbl +/+(WT) mice.

Comparison of response magnitude to a variety of taste, tactile, and thermal stimuli.

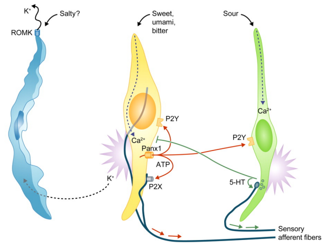
Finger, et al. Science 310, 1495 (2005);

Knockout of ATP receptors (P2X2 & P2X3) abolishes behavioral responses



Finger, et al. Science 310, 1495 (2005);

Synaptic, non-synaptic, and indirect activation of aff. nerve fibers



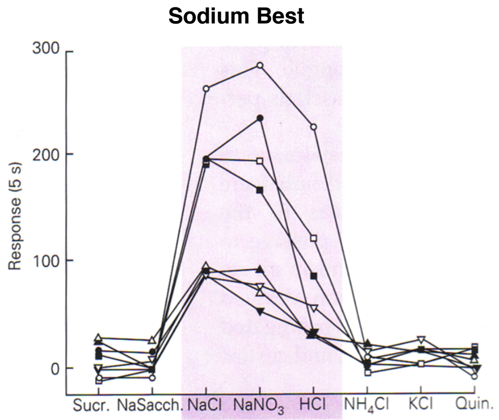
Type I glial-like cell	
Neurotransmitter clearance	
GLAST	Glutamate reuptake
NTPDase2	Ecto-ATPase
NET	Norepinephrine uptake
Ion redistribution and transport	
ROMK	K⁺ homeostasis
Other	
OXTR	Oxytocin signaling?

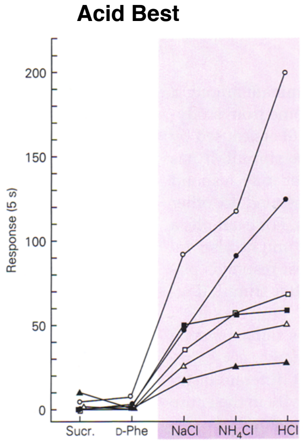
Type II receptor cell	
Taste transduction	
T1Rs, T2Rs	Taste GPCRs
mGluRs	Taste GPCRs
Gα-gus, Gγ13	G protein subunits
PLCβ2	Synthesis of IP3
TRPM5	Depolarizing cation current
Excitation and transmitter release	
$Na_v1.7$, $Na_v1.3$	Action potential generation
Panx1	ATP release channel

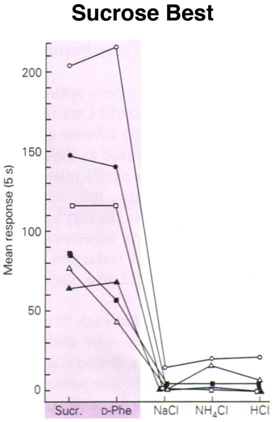
Type III presynaptic cell	
Surface glycoproteins, ion channels	
NCAM	Neuronal adhesion
PKD channels	Sour taste?
Neurotransmitter synthesis	
AADC	Biogenic amine synthesis
GAD67	GABA synthesis
5-HT	Neurotransmitter
Chromogranin	Vesicle packaging
Excitation, transmitter release	
$Na_v1.2$	Action potential generation
$Ca_v2.1$, $Ca_v1.2$	Voltage-gated Ca^{2+} current
SNAP25	SNARE protein, exocytosis

Chaudhari N, Roper S. JCB 2010;190:285-296

Gustatory Fiber Recordings


Sodium Best


Acid Best


Sucrose Best

Smith and Beidler Graph?

Neurotrophic Factors, Taste Cells and Gustatory Nerves

Taste cells produce neurotrophins (e.g. BDNF)

Gustatory nerves have BDNF (trk3) receptors

Neurotrophic factor brings nerves to taste bud

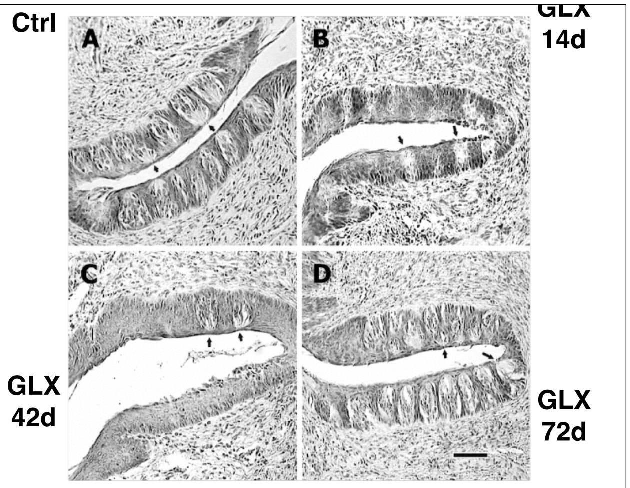
Presence of nerve maintains taste bud.

Ongoing process because of turnover of taste buds
(every 2 weeks)

Loss of Gustatory Nerve -> Loss of Taste Buds Regeneration of Nerve -> Regrowth of Taste Buds

Transection of Glossopharyngeal Nerve ->
Loss of taste buds in circumvallate papillae
(regrowth after regeneration)

Transection of Greater Superficial Petrosal Nerve ->
Loss of taste buds on soft pallete
(but no regrowth)



Taste Buds return in a linear fashion as Glossopharyngeal Nerve regenerates