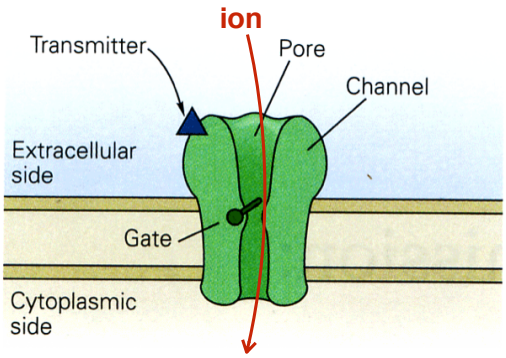
Metabotropic Receptors and Second Messengers

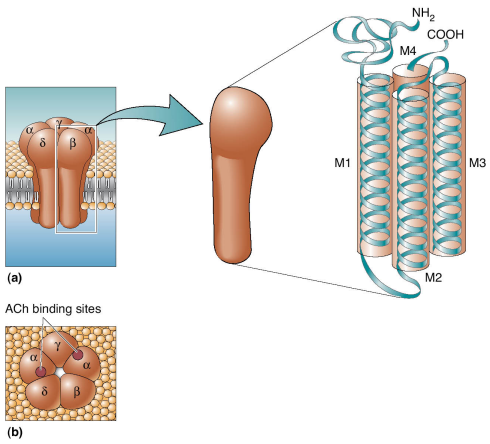
There are 2 classes of neurotransmitter receptors:

1. **ionotropic**
   - **NT causes opening of ion channel**
   - **differentiates between ions**
   - **rapid, electrochemical effect:**
   - **direct effect on V_m**
2. **metabotropic**
   - **NT starts enzymatic cascade**
   - **leads to slower, intracellular effects**
   - **indirect effect on V_m**

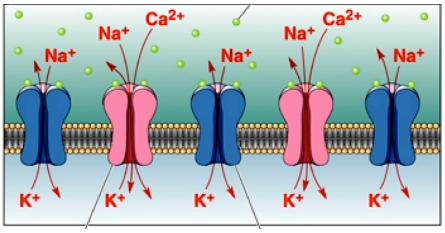
Direct, ionotropic receptor

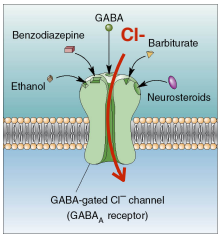


Subunit Arrangement of Nicotinic Ach Receptor

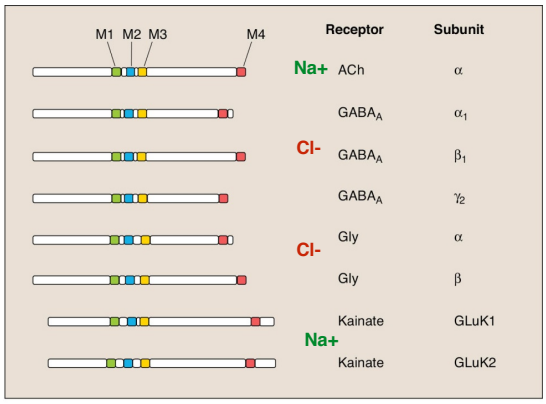


- Glutamate-gated Na+ channels
  - AMPA, NMDA, kainate
- GABA-gated Cl- channel





Similarities in Structure of Subunits for Different Transmitter-Gated Ion Channels



2 types of Metabotropic Receptors

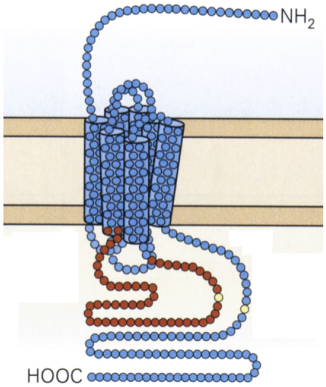
1. G-protein coupled receptors
NT causes receptor to activate other enzymes to cause second messenger synthesis

2. receptor tyrosine kinases
The receptor itself is an enzyme that is activated by NT
the receptor phosphorylates itself and other substrates.

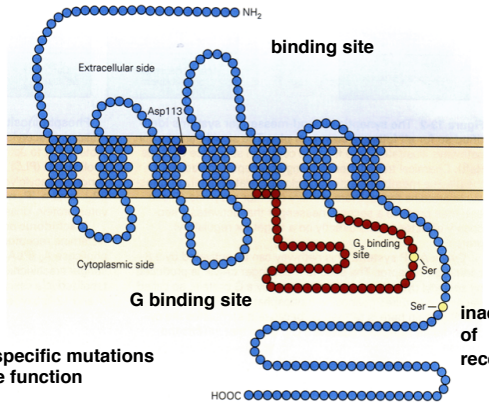
G-protein coupled receptors (GPCRs)

- **Three steps in transmission**
  - Binding of the neurotransmitter to the receptor protein
  - Activation of G-proteins
  - Activation of effector systems (which may be inhibitors)
- **Basic structure of G-protein-coupled receptors (GPCRs)**
  - Single polypeptide with 7 membrane-spanning alpha-helices
- Different receptor types activate different types of G-proteins (eg G-stimulatory, G-inhibitory)

G-protein coupled receptors have 7-transmembrane domains



G-protein coupled receptors have 7-transmembrane domains



binding site

G binding site

inactivation of receptor

Use site-specific mutations to analyze function

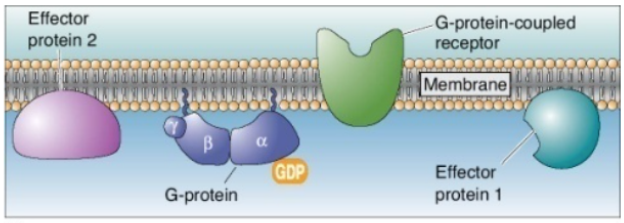
cytoplasmic tail

Five Steps in G-Protein Operation

- Inactive: 3 subunits—α, β, and γ—"float" in membrane (α bound to GDP)
- Active: bumps into activated receptor and exchanges GDP for GTP
- G_{α}-GTP and $G_{\beta\gamma}$—influence effector proteins
- G_{α} inactivates by slowly converting GTP to GDP.
- G_{α} and $G_{\beta\gamma}$ recombine to start the cycle again.

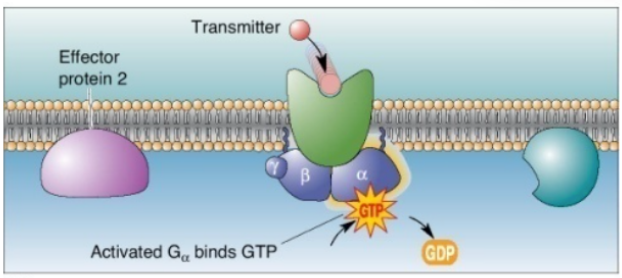
1 activated receptor
-> many G proteins
-> many other effector proteins

- Inactive: 3 subunits—α, β, and γ—"float" in membrane (α bound to GDP)



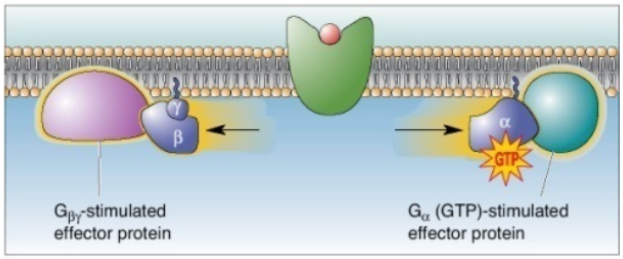
(a)

- Active: bumps into activated receptor and exchanges GDP for GTP



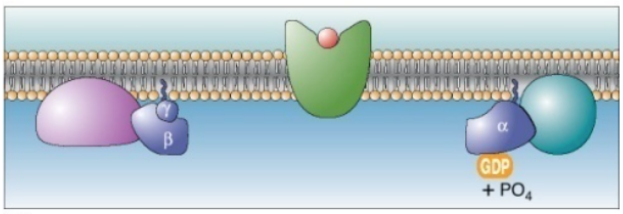
(b)

- G_{α}-GTP and $G_{\beta\gamma}$—influence effector proteins



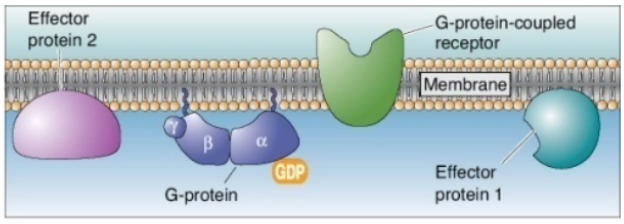
(c)

- G_{α} inactivates by slowly converting GTP to GDP.



(d)

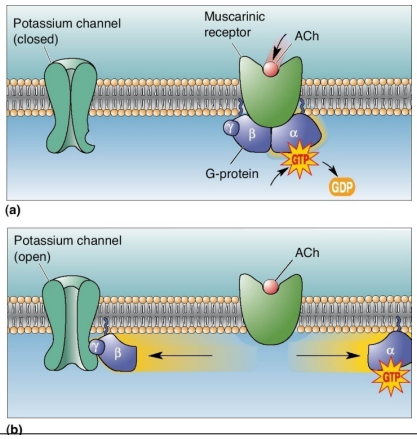
- G_{α} and $G_{\beta\gamma}$ recombine to start the cycle again.



(a)

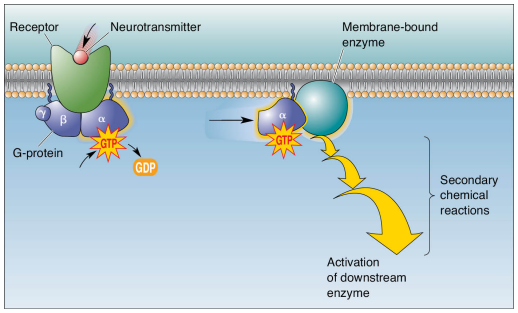
G-protein coupled receptor effector systems

Shortcut: from receptor to G-protein to ion channel—fast and localized



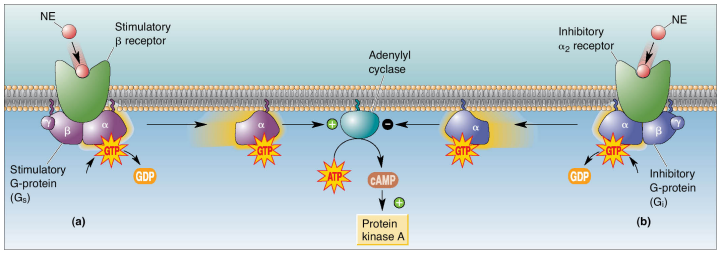
G-protein coupled receptor effector systems

Second messenger cascades: G-protein couples neurotransmitter with downstream enzyme activation

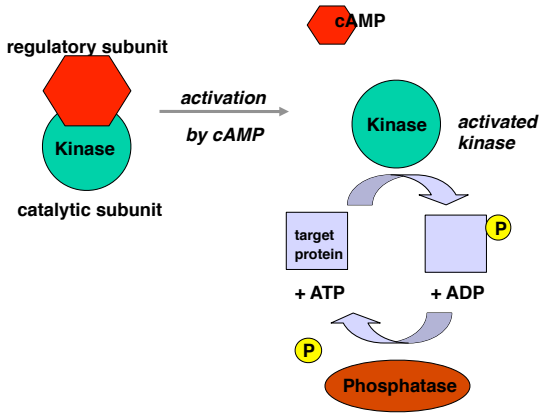


G-protein coupled receptor effector systems

Push–pull: different G-proteins stimulate or inhibit adenylyl cyclase

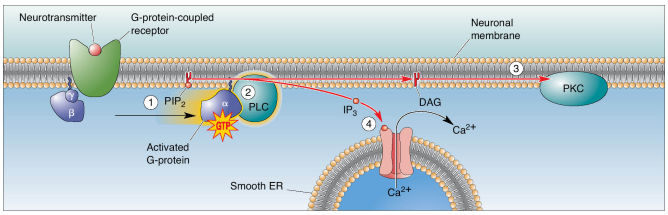


Protein Kinase A and Phosphotases



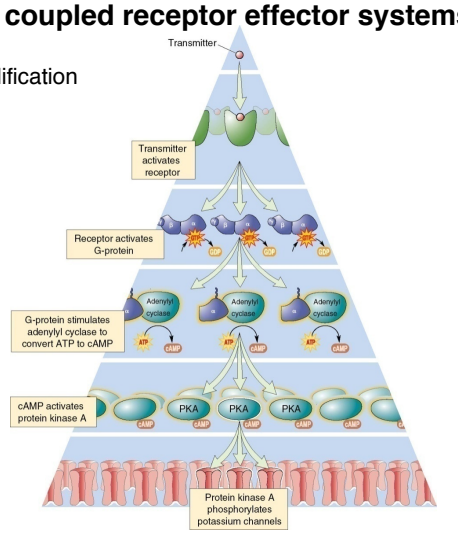
G-protein coupled receptor effector systems

Some cascades branch
G-protein activates PLC -> DAG, IP_3 -> activate different effectors



G-protein coupled receptor effector systems

Signal amplification



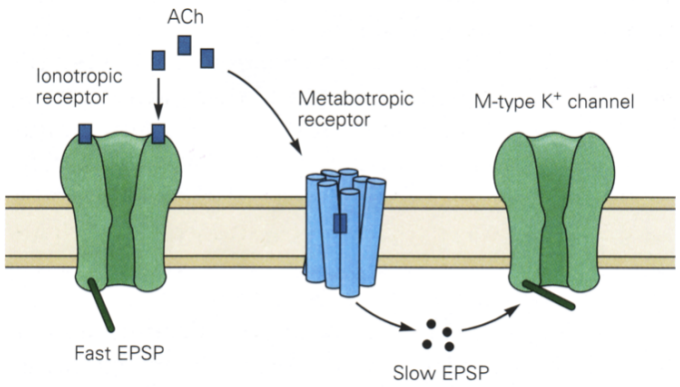
G-protein Toxins

1. **Cholera Toxin** -- allows binding of GTP, but prevents hydrolysis.

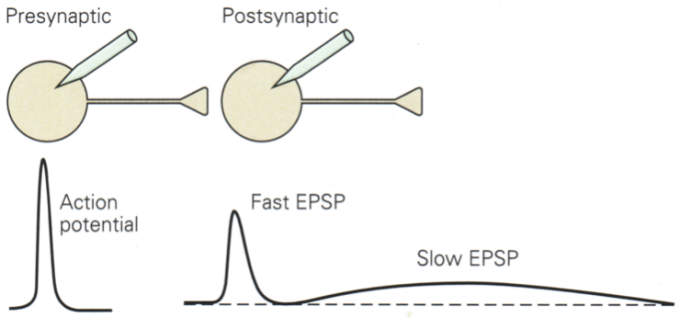
   Causes overproduction of cAMP, leading to loss of electrolytes and water from intestinal cells.

2. **Pertussis Toxin** -- blocks release of GDP from alpha subunit, so G-protein locked in the inactive state.

The same neurotransmitter can act at both ionotropic and metabotropic receptors



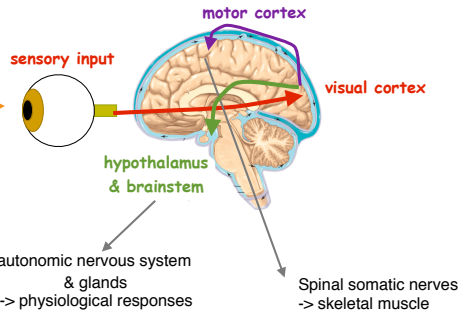
The same neurotransmitter can act at both ionotropic and metabotropic receptors



General Scheme of CNS

receive sensory inputs, coordinate response of the organs & functions of the body

inputs:
see bear -> retina -> cranial nerve II (optic nerve) -> visual cortex
outputs:
-> motor cortex -> run away
-> hypothalamus -> stress hormone release -> mobilize glucose
-> brainstem -> increase heart rate, blood pressure, breathing



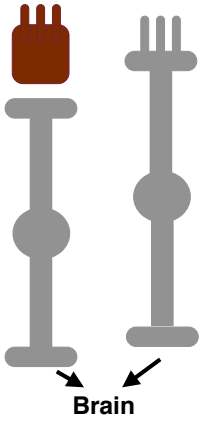
autonomic nervous system
& glands
-> physiological responses

Spinal somatic nerves
-> skeletal muscle

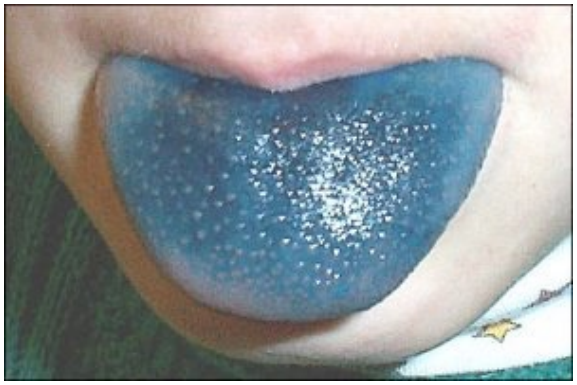
Sensory Systems

taste, smell, vision, hearing/vestibular, somatosensation

1. Nature of Stimuli
2. Receptor Cells
3. Sensory Neurons
   - Fibers in Nerves
   - Cell bodies in Ganglia
4. First Central Relay
   - e.g. spinal cord, brainstem
5. Central Representation
   - how the brain encodes the sensory information
6. Control of Motor Responses

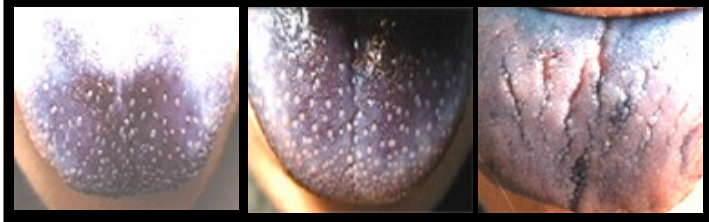


Methylene Blue staining of taste buds

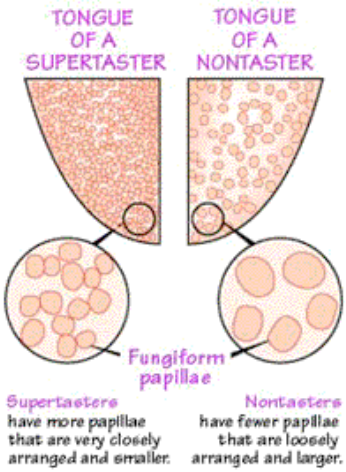


Colorado State ETextbook

Non-Taster | Medium Taster | Taster



Cornell Nutritional Sciences.



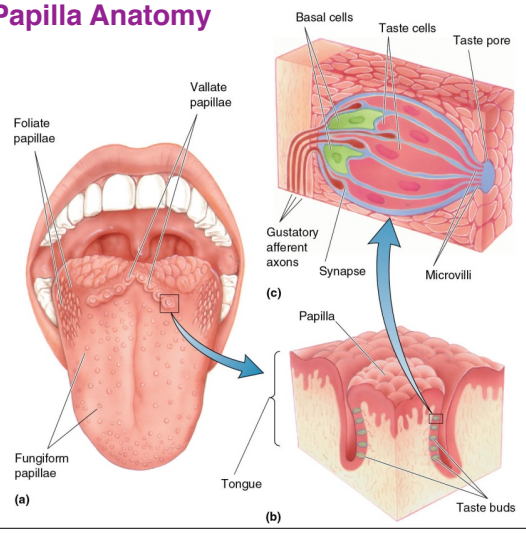
PROP sensitivity = polymorphism in bitter receptors

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

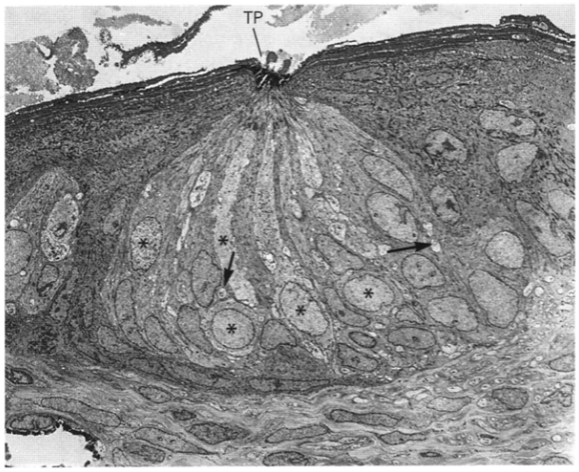
(propylthiouracil)

Scientific American Frontiers, www.pbs.org

Taste Papilla Anatomy



Taste cells and taste pores



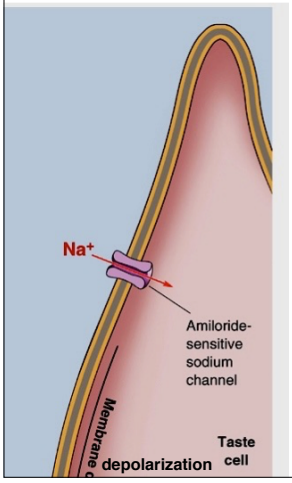
Taste Qualities

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

Non-Taste

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

Epithelial Sodium Channel (ENaC)

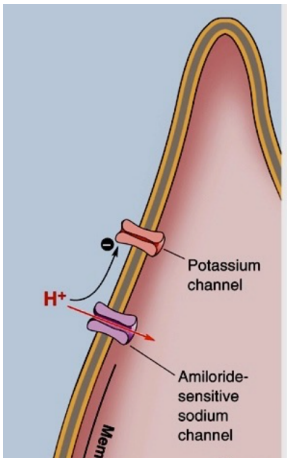


allows Na, Li, K to enter cell -> depolarization

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

Blocked by amiloride (ENaC blocker)

Sour Taste through H^+ , K^+ channels



Hydrochloric acid, citric acid, ammonia

H^+ passes through ENaC or K^+ channel, et al.

Isolation of Miraculin

A native shrub (*Synsepalum dulcificum*) in tropical West Africa yields a small, red berry that, once its pulp is chewed, causes sour substances to taste sweet. Local people often use it to make their stale and acidulated maize bread more palatable and to give sweetness to sour palm wine and beer. Daniell (*1*) first described the unusual properties of the berry and called it miraculous berry. Others call it miracle fruit.

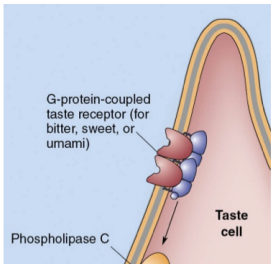
Kurihara & Beidler, Science 1968

The berries were grown in a greenhouse at the Florida State University. Since the active principle is labile, the berries were stored in a deep freezer (−70°C) until needed. The sweetening activity was assayed on four subjects. Five milliliters of a solution containing the active principle was kept in the mouth for 2 minutes and was spit out. The mouth was rinsed with distilled water, and 0.02*M* citric acid solution was tasted. For a quantitative measurement of the activity, the subject was asked to choose one out of a series of ten sucrose solutions (0.1 to 1.0*M*) which best approximated the intensity of sweetness of the given citric acid.

The purified protein itself has no inherent taste. A mixture of the protein with sour substances initially tastes sour, and slowly changes to sweet if the mixture is held in the mouth for about 1 minute. This fact ruled out the possibility that a complex of the protein with the sour substance itself has a sweet taste. It is believed that the protein binds to receptors of the taste buds and modifies their function. We call the protein "taste-modifying protein."

KENZO KURIHARA
LLOYD M. BEIDLER
Department of Biological Science, Florida State University, Tallahassee

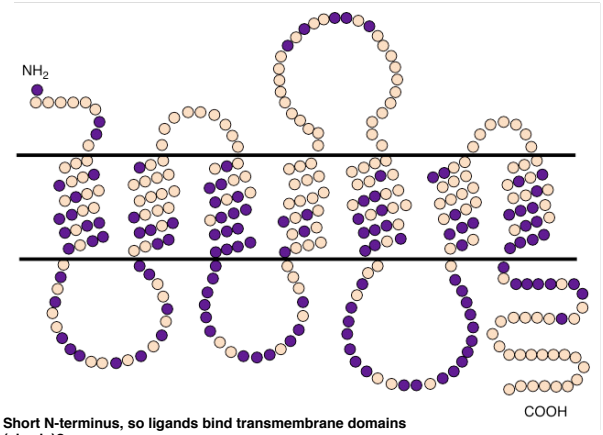
T2Rs Bitter Taste Receptors



Quinine - prototypical bitter taste
alkaloids - common bitter poisons
some species tolerate bitter tastes

- **Mediate bitter taste**
- **~26 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**

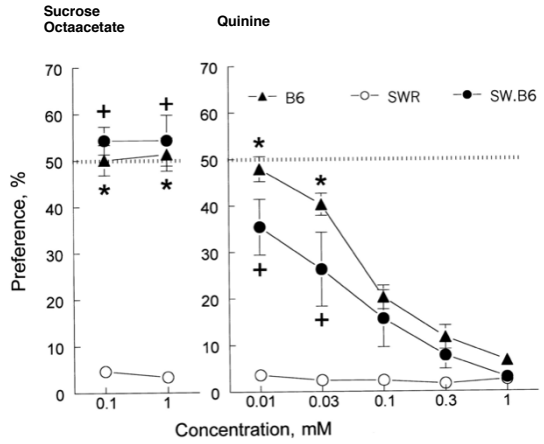
T2R Bitter receptor



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

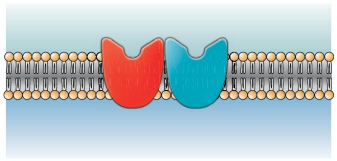
Montmayeur 2002

SOA gene locus (one of T2R receptors) influences bitter taste



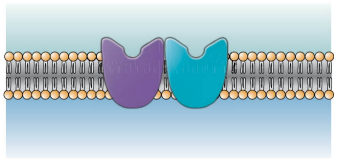
T1Rs Sweet & Umami Taste Receptors expressed as hetereodimers

Sweet receptor: T1R2 + T1R3



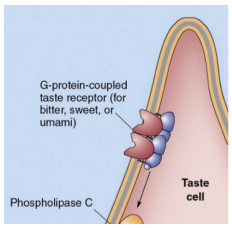
(b)

Umami receptor: T1R1 + T1R3



(c)

Sweet Taste T1R2 & T1R3 dimer receptor



Sucrose - prototypical sweet taste (all animals* love sweet)

some amino acids (phenylalanine)

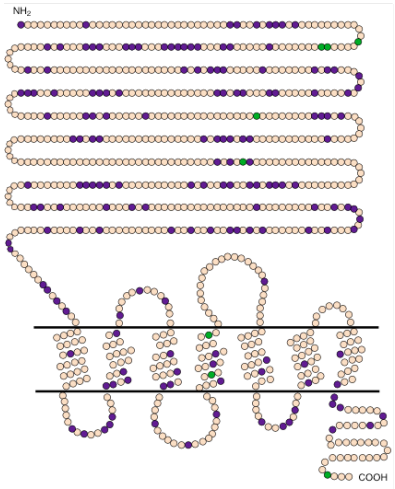
Artificial sweetners:

- **saccharin (lo conc)**
- **cyclamate**
- **aspartame**

(detection of sweetners is species specific)

T1R3 sweet receptor

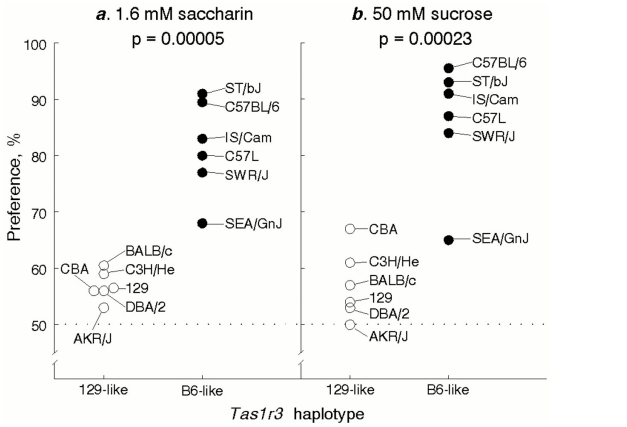
Large N-terminus so extracellular ligand binding



Green = Sac polymorphisms

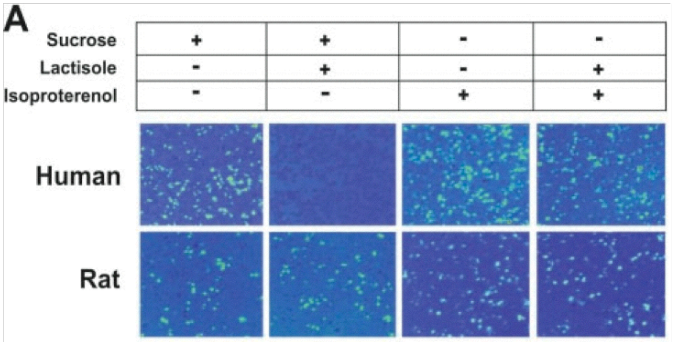
Blue = T1R common a.a.

Sac Locus and Sweet Preference in Mouse Strains



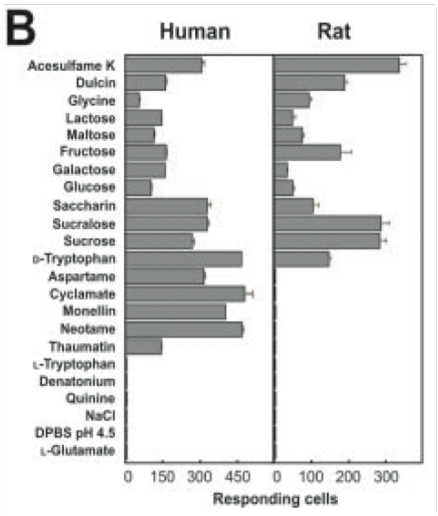
Bachmanov Chem Sen 2001

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



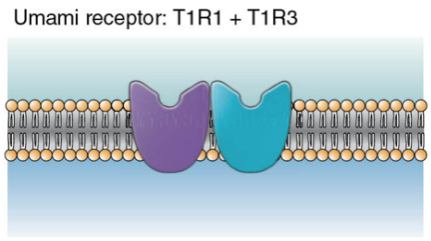
lactisole = sweet inhibitor for humans
isoproterenol = stimulates adrenergic G-protein coupled receptors

Li et al, PNAS 2002

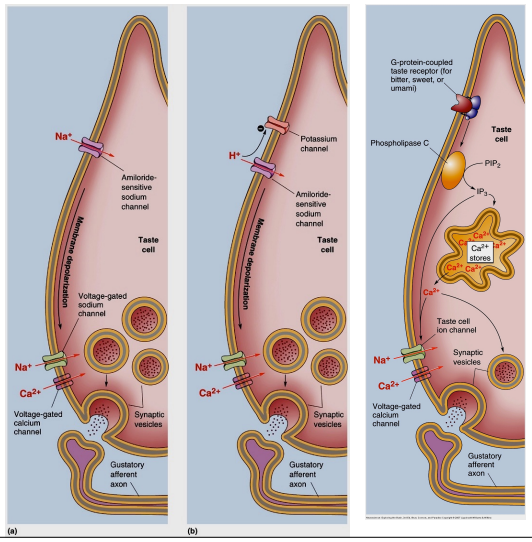


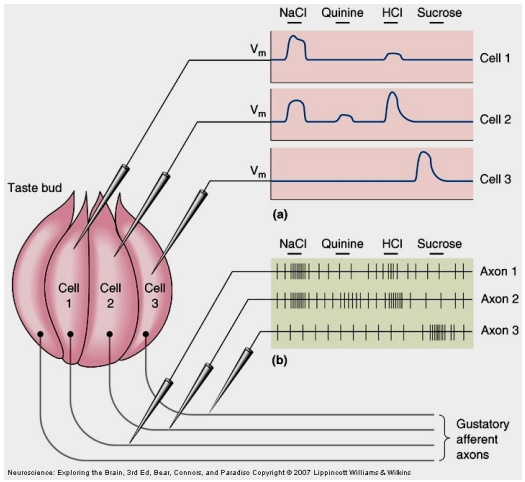
Unami Taste and Glutamate Receptors

L-Amino acids detected by heterodimers of T1R3 & T1R1

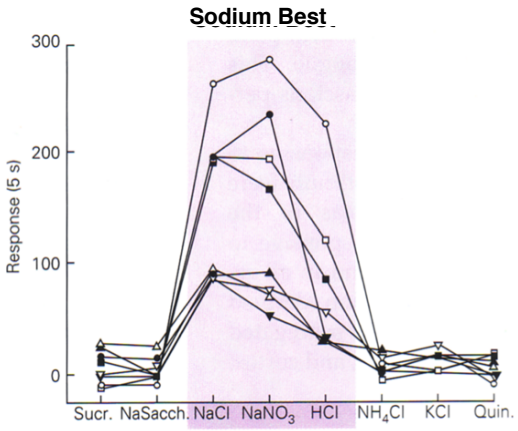


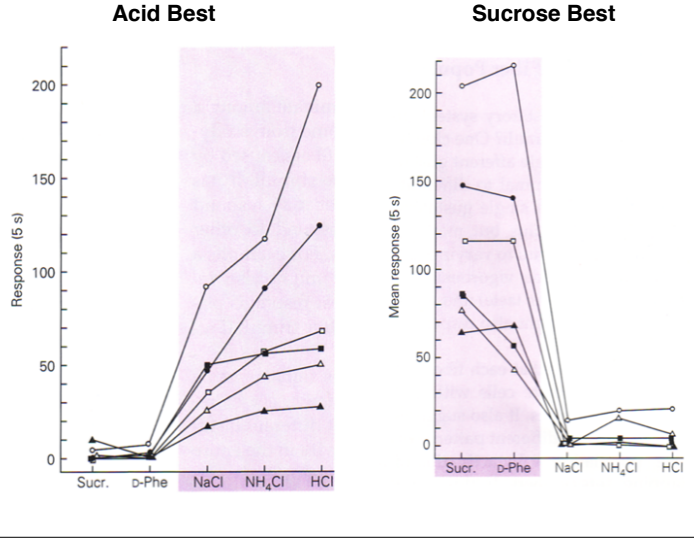
(c)



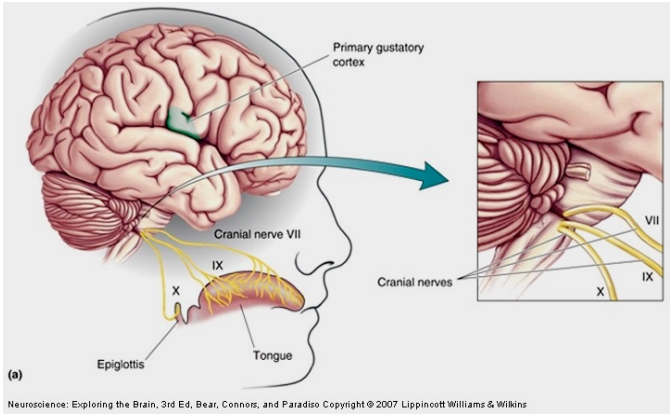


Gustatory Nerve Fiber Recordings: example of "labeled line" coding

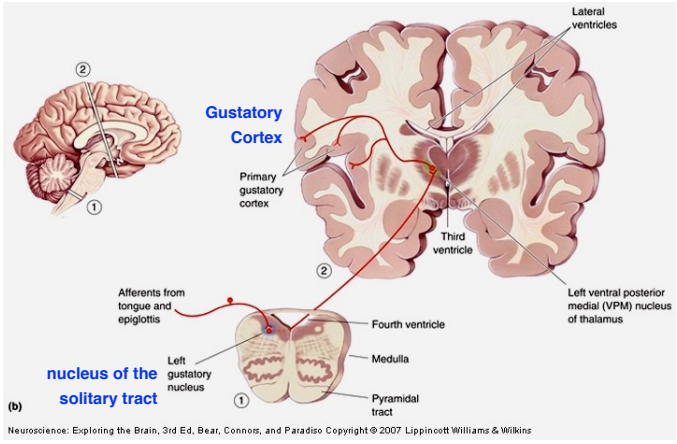




Central Taste Circuit



Central Taste Circuit



Brainstem as "spinal cord" for taste input

Brainstem mediates "reflex" like behavioral and physiological responses to tastants:

sweet, lo salt -> ingestive responses
licking, swallowing

bitter, sour, hi salt -> aversive responses
spitting, vomiting or gaping

sweet -> insulin release from pancreas
cephalic insulin response

Taste Reactivity

measure orofacial responses to taste stimuli infused directly into mouth.

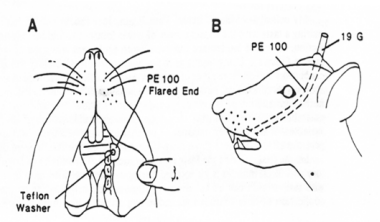
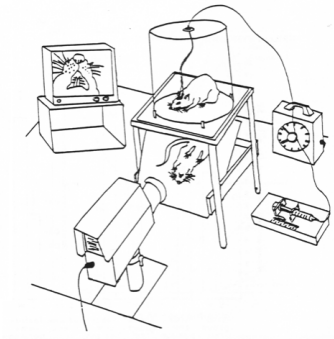


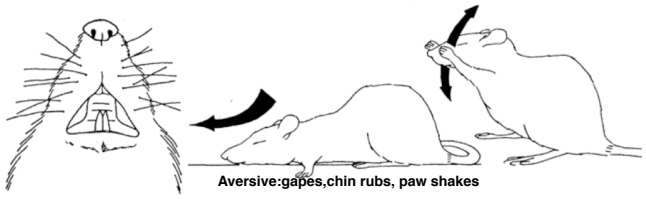
FIGURE 3. Diagram of the intraoral catheter. The intraoral end is placed just rostral to the first maxillary molar. The tubing is led out subcutaneously to the skull and secured to a short piece of 19-gauge (19 G) stainless steel tubing with dental acrylic. (A) Ventral view. (B) Lateral view.



Taste Reactivity as "reflex" response

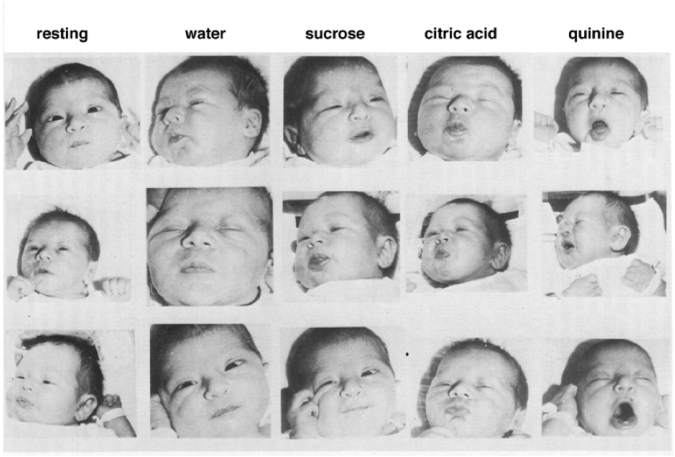
Stereotyped orofacial movements of the rat when mouth infused with tastants - scored with slow motion videotapes

Ingestive: mouth movements, tongue protrusions

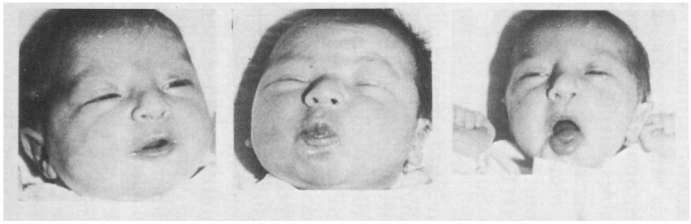


Aversive:gapes,chin rubs, paw shakes

Behavioral Responses to Taste are innate:

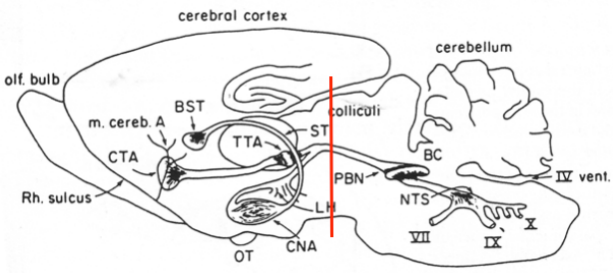


sucrose citric acid quinine



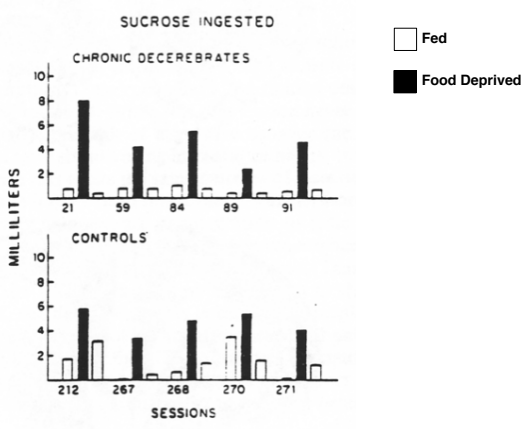
Newborns receiving tastants within minutes of birth:
sucrose elicits mouth smacking, swallowing, smiles
quinine elicits spitting, grimaces, crying

Hindbrain alone can generate behavioral responses

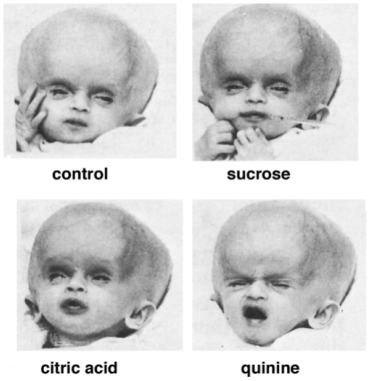


decerebrate rat with only hindbrain intact

Decerebrate Rats respond normally to Palatable Tastants
(also to aversive tastants, not shown)



Human Hindbrain alone can generate behavioral responses



control sucrose

citric acid quinine

hydroanencephalic babies with essentially no forebrain but intact hindbrain have same responses