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Connection with broader issues of physiology

Bioinformatics*:* **The process and methods applied to the upgrade of the information content of biological measurements**

This includes whole animal and human physiological data.

Books:

Molecular Evolution :A Phylogenetic Approach (1998) by Roderic D. M. Page, Edward C. Holmes.

Blackwell Science Inc; ISBN: 0865428891

Easy read, nice introduction

Molecular Evolution (1997) by Wen-Hsiung Li

Sinauer Assoc; ISBN: 0878934634

More Detailed than Page and Holmes

Human Molecular Genetics (1999) by Tom Strachan, Andrew P. Read

Wiley-Liss; ISBN: 0471330612

Well written human genetics text

Bioinformatics: Sequence and Genome Analysis by David W. Mount

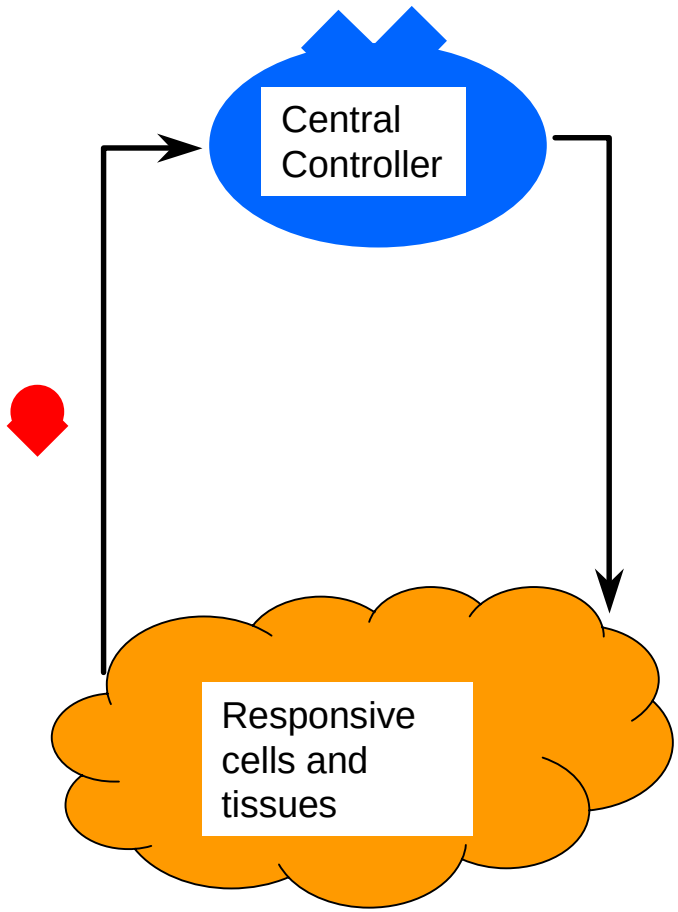
Cold Spring Harbor Laboratory; ISBN: 0879696087

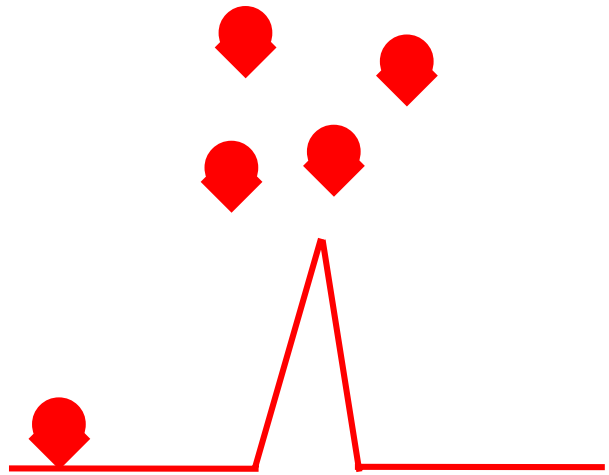
Basic Bioinformatics Text - Easy to follow for Biologist

Important web site not previously mentioned in the course is OMIM

Online Medelian Inheritance in Man **http://www3.ncbi.nlm.nih.gov/omim/**

The central concept in Human Physiology is Homeostasis, maintaining a constant internal environment. Classic feedback loops are responsible for many homeostatic mechanisms.





Disease may result from

Defective Hormone

Defective Receptor

Inappropriate set point for Central Controller

Endocrine and Metabolic Physiology concerns blood borne metabolites and hormones

Peptide hormones communicate with cells via membrane receptors.

Steroid hormones and other small molecules freely enter cells and act intracellularly

Discover of Insulin 1921, from Physiology to Gene

1889. It was known that removal of pancreas from dog resulted in diabetes, high blood sugar

Minkowski hypothesized that a substance from the pancreas was required to lower blood glucose after a meal. But efforts to isolate this substance failed.

Banting hypothesized that enzymes had destroyed this substance in previous work.

1921. Banting, Best and Collip purified a glucose lowering substance from dog pancreas.

1922 insulin was successfully used to treat patients.

1970. Radio-immunoassays made possible the detection and estimates of plasma glucose.

Later, the insulin gene and insulin receptor genes were identified and cloned.

1982. Humulin, recombinant human insulin became the first human therapeutic protein marketed in the United States.

Question for Metabolic Physiologists in the 1980s: Is there a fat hormone?

Arguments against: a new hormone

No major circulating hormones feed back loops discovered recently.

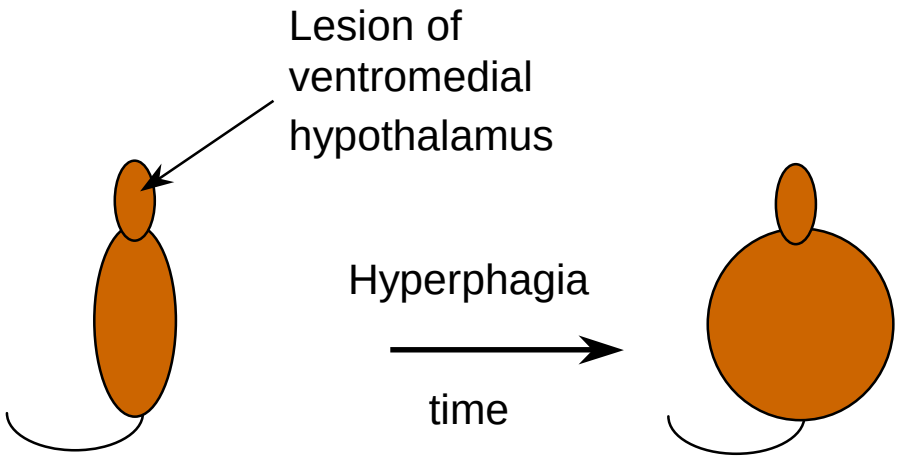
Insulin, the carbohydrate hormone discovered in and purified in 1921.

Radio immunoassays developed in late 1970's finally allow measuring concentrations of hormones nM or pM in plasma. We understand hormones at last.

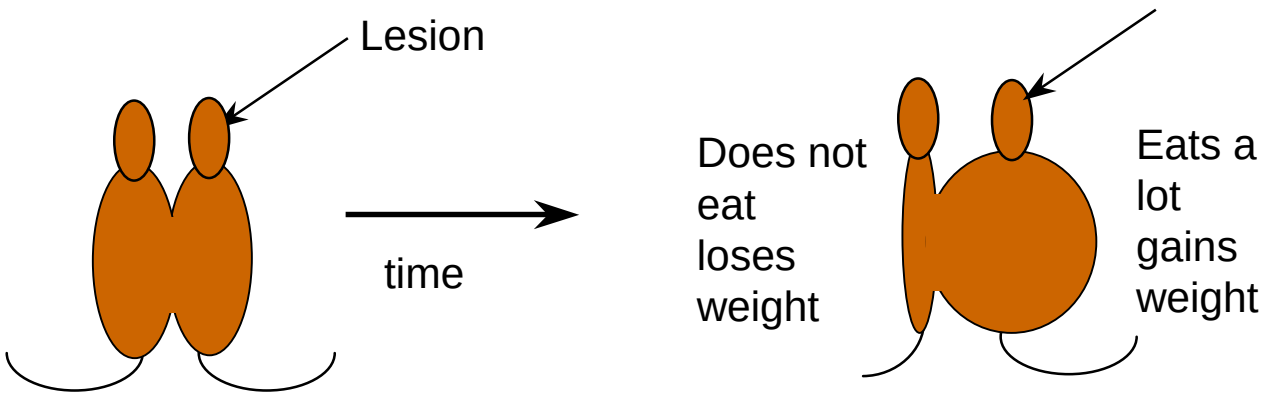
Arguments for a new hormone

Common sense: A theoretical 155-pound man, eats about a million calories a year and burns about a million calories a year. To gain a pound a year, you only have to be off by 10,000 calories - only one percent To gain a pound you only have to eat 27 calories a day more than you burn. That's a fraction of a cookie'

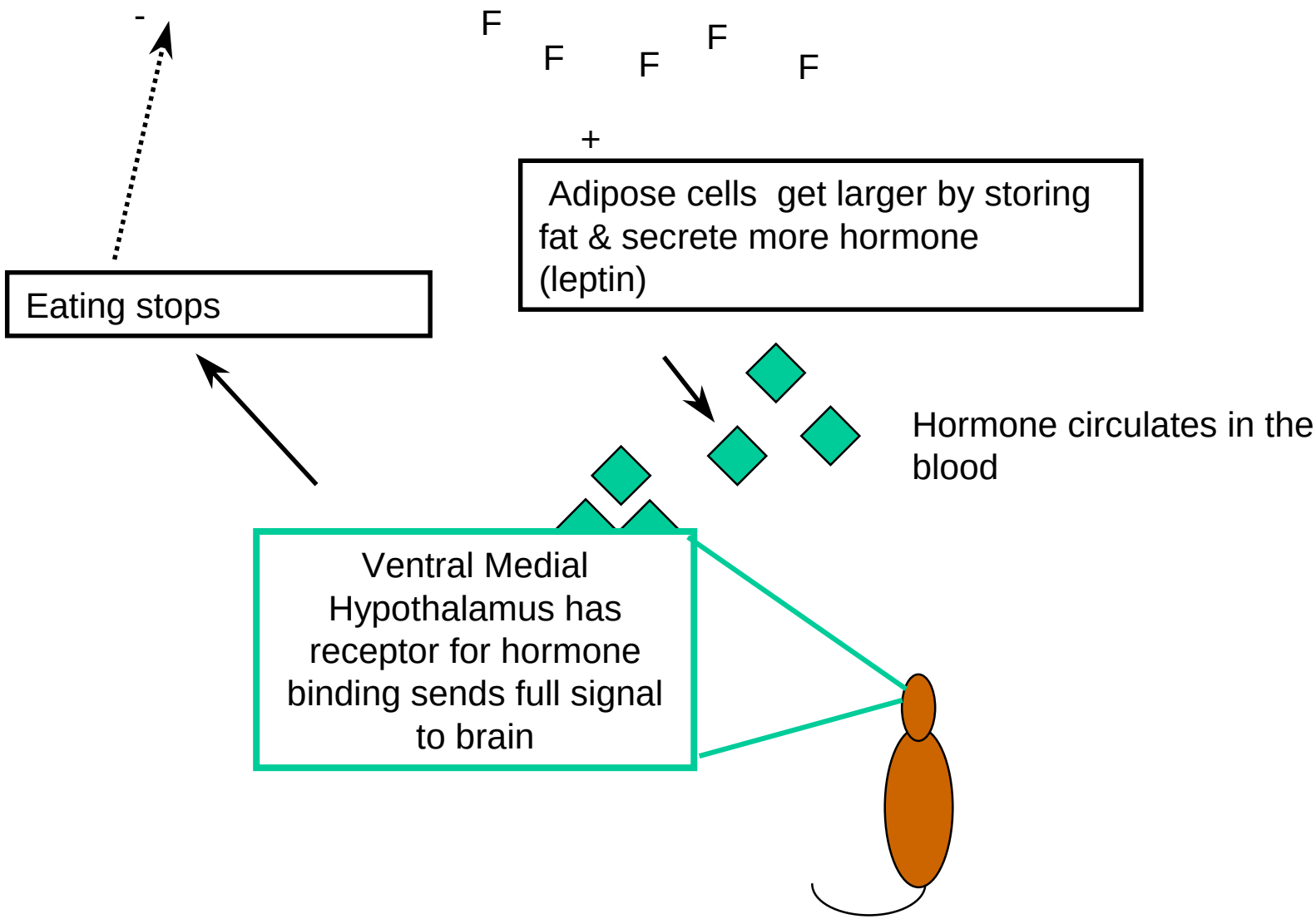
Classic physiology



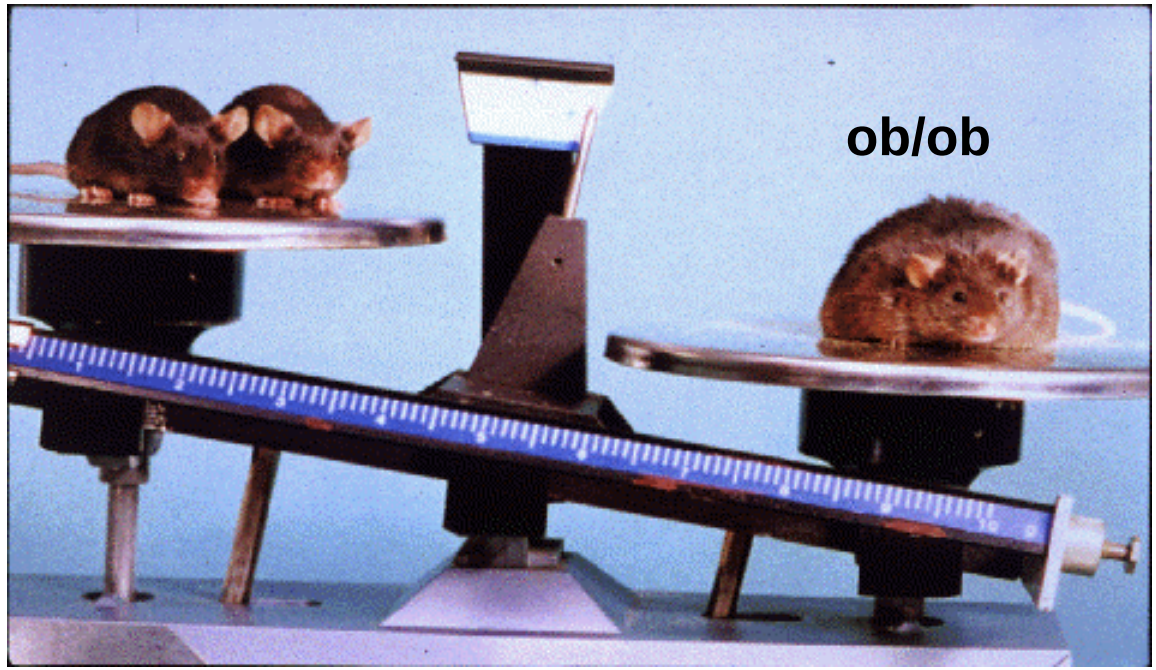
Parabiotic mice: connected by common microvasculature – slow exchange of compounds between two animals.



Fat enters the GI track after a meal



ob/wt
wt/wt



Note: mammalians are diploid

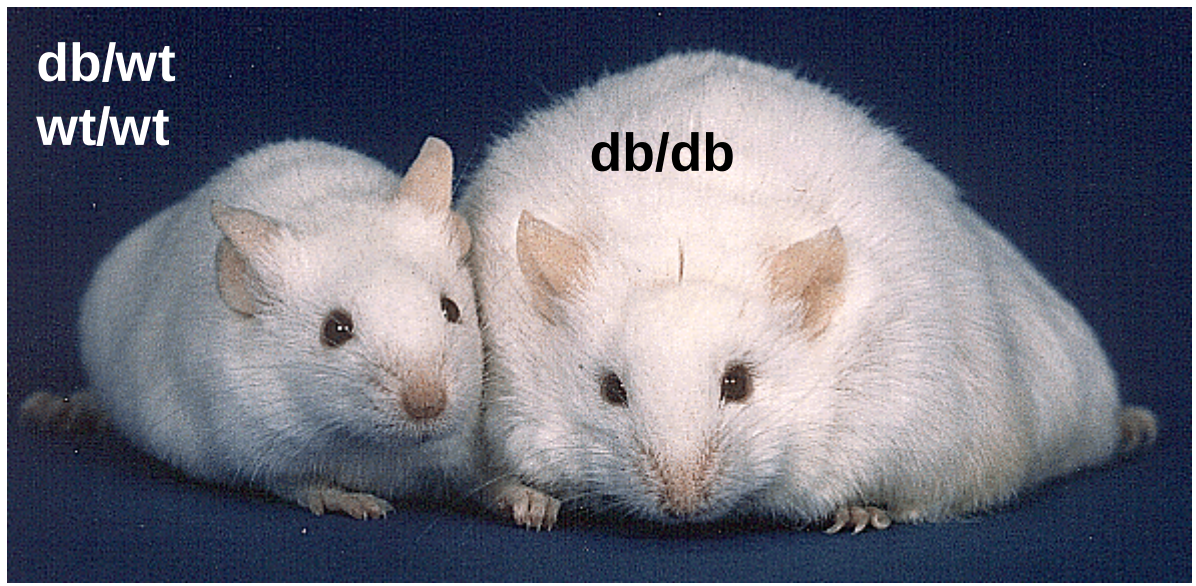
Two copies of each gene

These mutants are recessive

Homozygous mutant animals

ob/ob and db/db are:

infertile obese and diabetic



Discover of Leptin 1994, from Gene to Physiology

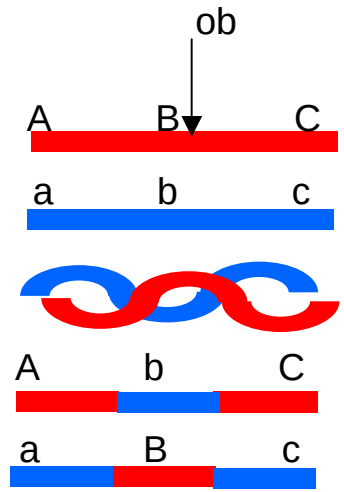
linkage analysis mapped 'ob" to mouse chromosome 6 and suggested that human homolog would be on chromosome 7

mouse breeding and RFLP analysis narrowed the region on chromosome 6

positional cloning of ob compared to wt finally found the gene

protein sequence was immediately known

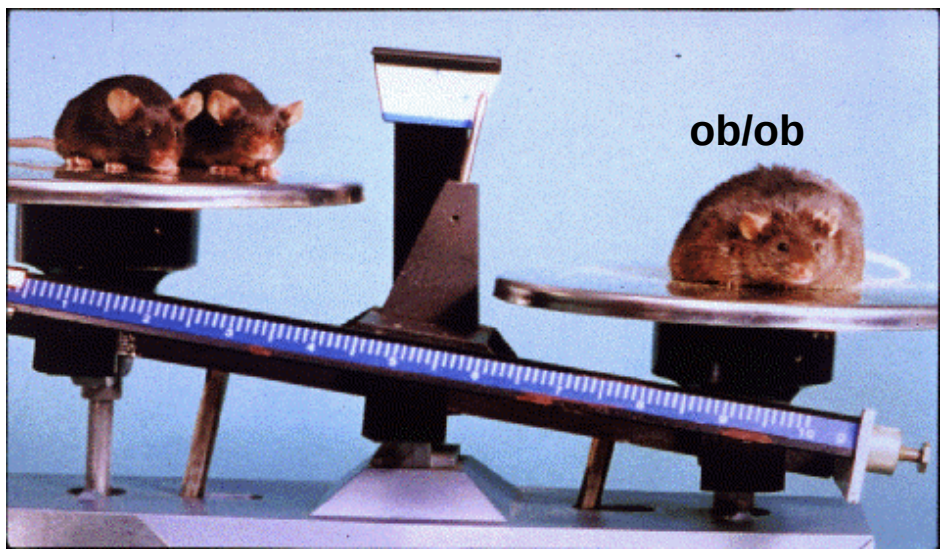
RIA developed plasma levels measured



Lesson: Trust Physiology

ob/wt

wt/wt

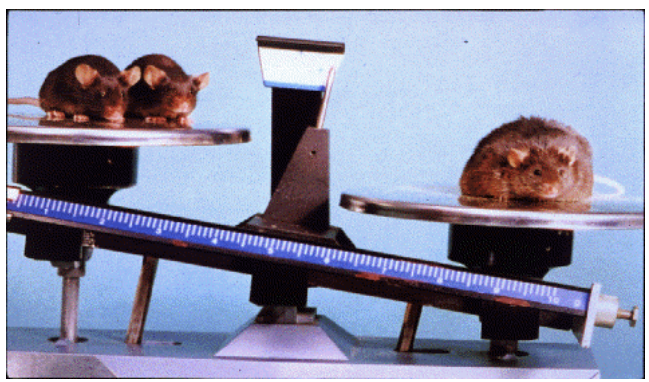


Y. Zhang, R. Proenca, M. Maffei, M. Barone, L. Leopold, and J. M. Friedman. 1994. Positional cloning of the mouse obese gene and its human homologue. *Nature* 372(6505):425-432

Ob mouse: Adipose cells secrete mutant hormone that cannot bind receptor

Eating does not stop

Ventral Medial Hypothalamus has normal receptor for hormone but no binding - no signal to brain



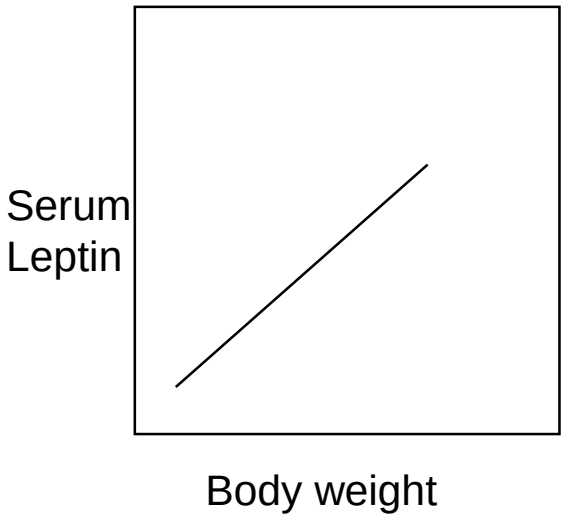
Ob mouse

Eating does not stop

Adipose cells secrete Normal hormone

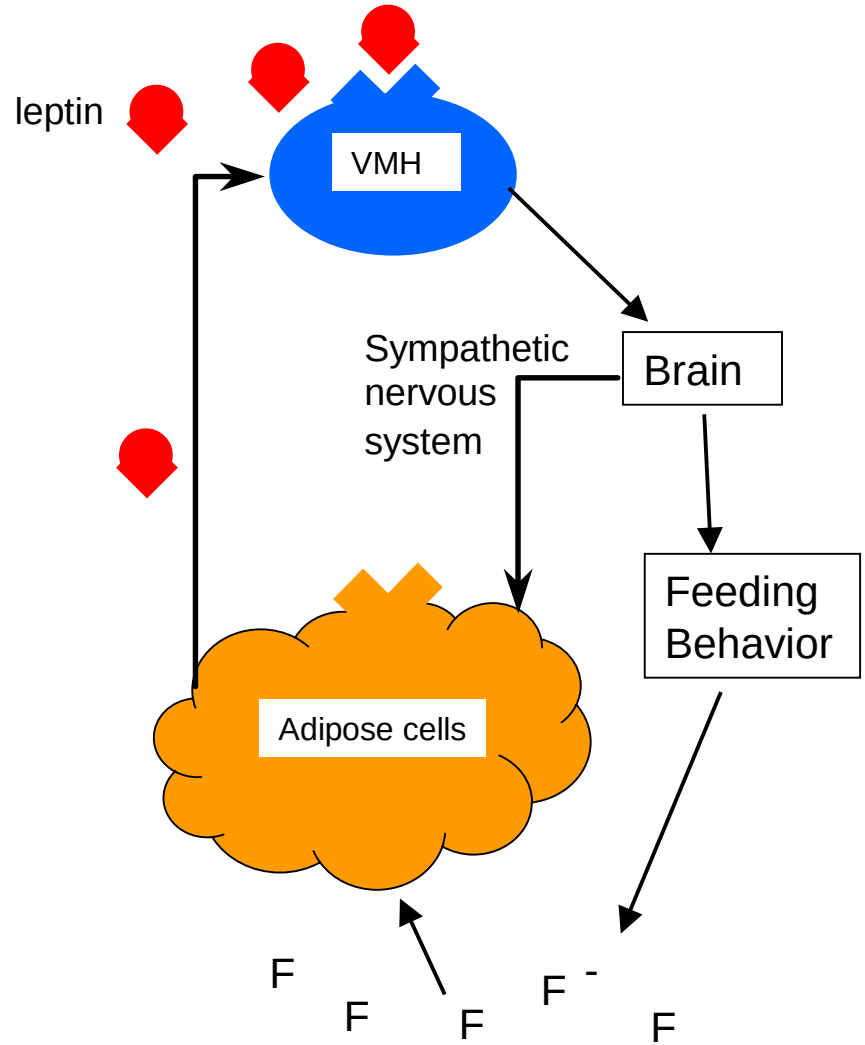
Db mouse: Ventral Medial Hypothalamus has mutant receptor for hormone no binding - no signal to brain

Db mouse



Unlike Insulin and Diabetes,

Severe Defects in Leptin gene or Leptin receptor are rare in humans



Discover of Leptin 1994, from Gene to Physiology

A new hormone was discovered in 1994

Why was it hard to find.

secreted by fat cells 20- 30 % body weight vs pancreas < 1%

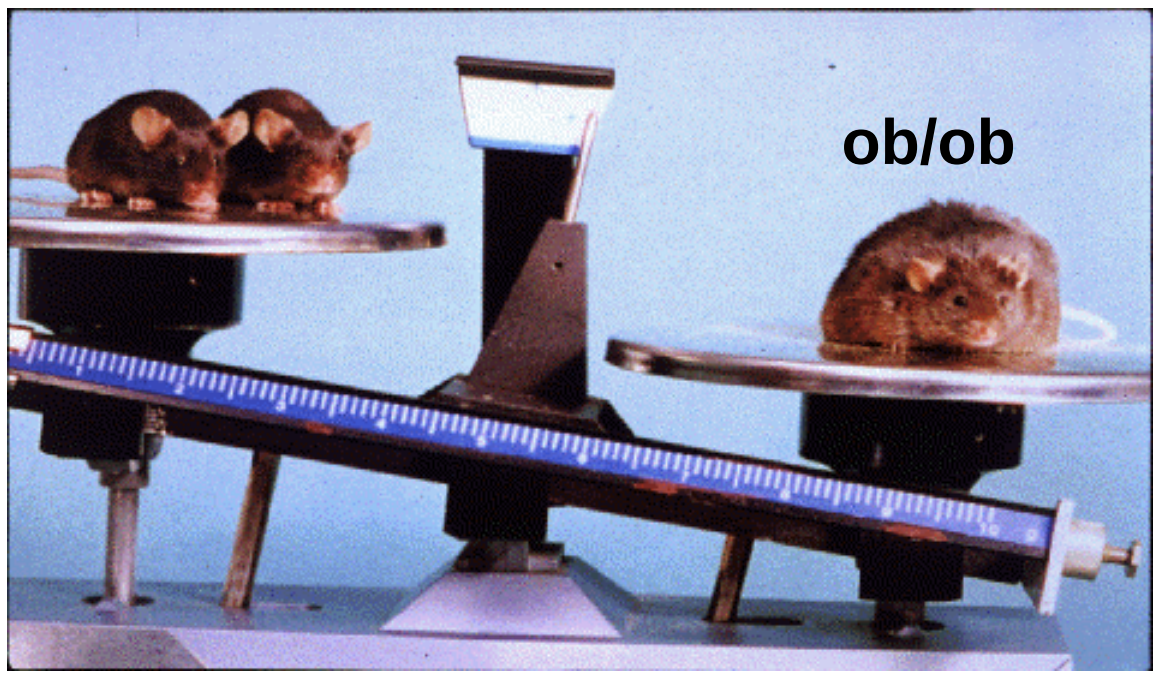
present at low concentration in blood about 0.3 nM binds to receptor kd about 0.3 nM

major receptor is tiny part of brain: ventral medial hypothalamus

Lessons

Trust physiology

ob/wt
wt/wt



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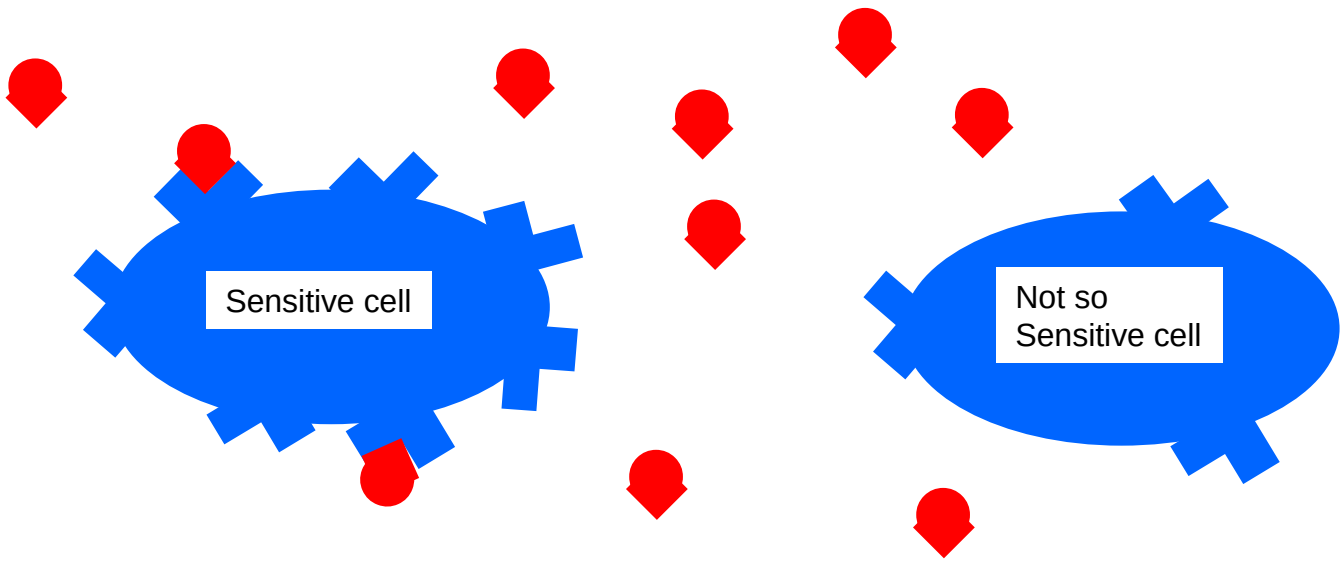
Peptide hormone receptor binding.

often sensitivity of cell depends on number of receptors

Hormone concentration in plasma low, leptin level around 0.3 nM

Relatively high affinity binding Kd may be about 0.3 nM

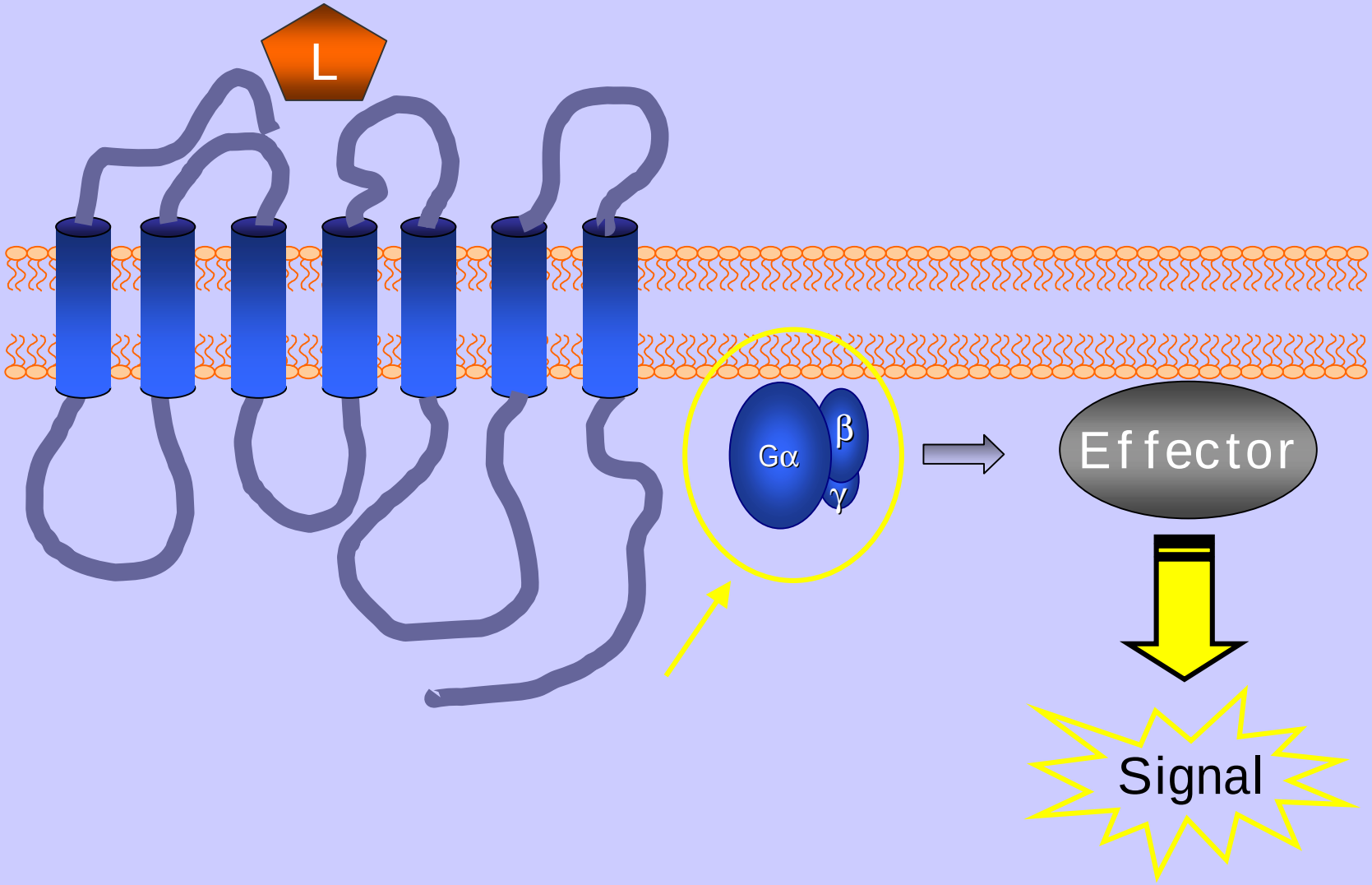
In sensitive cells only a small fraction of receptor must be bound for max physiological effect



Can one Learn from Mistakes

A new opportunity to bet on the future.

G- Protein Coupled Receptors



Why is the pharmaceutical industry interested in oGPCRs?

A. GPCRs are good drug targets

✓ 50% of prescription drugs interact with GPCRs

- Hypertension
- Stomach ulcers
- Migraine
- Allergies

B. GPCRs in disease states

✓ Disease states associated with GPCR mutations

- Rhodopsin receptor → retinitis pigmentosa
- Vasopressin V2 → nephrogenic diabetes
- Glucagon → diabetes, hypertension

How many GPCRs are encoded by the human genome?

✓First estimation based on comparison with *C. elegans*

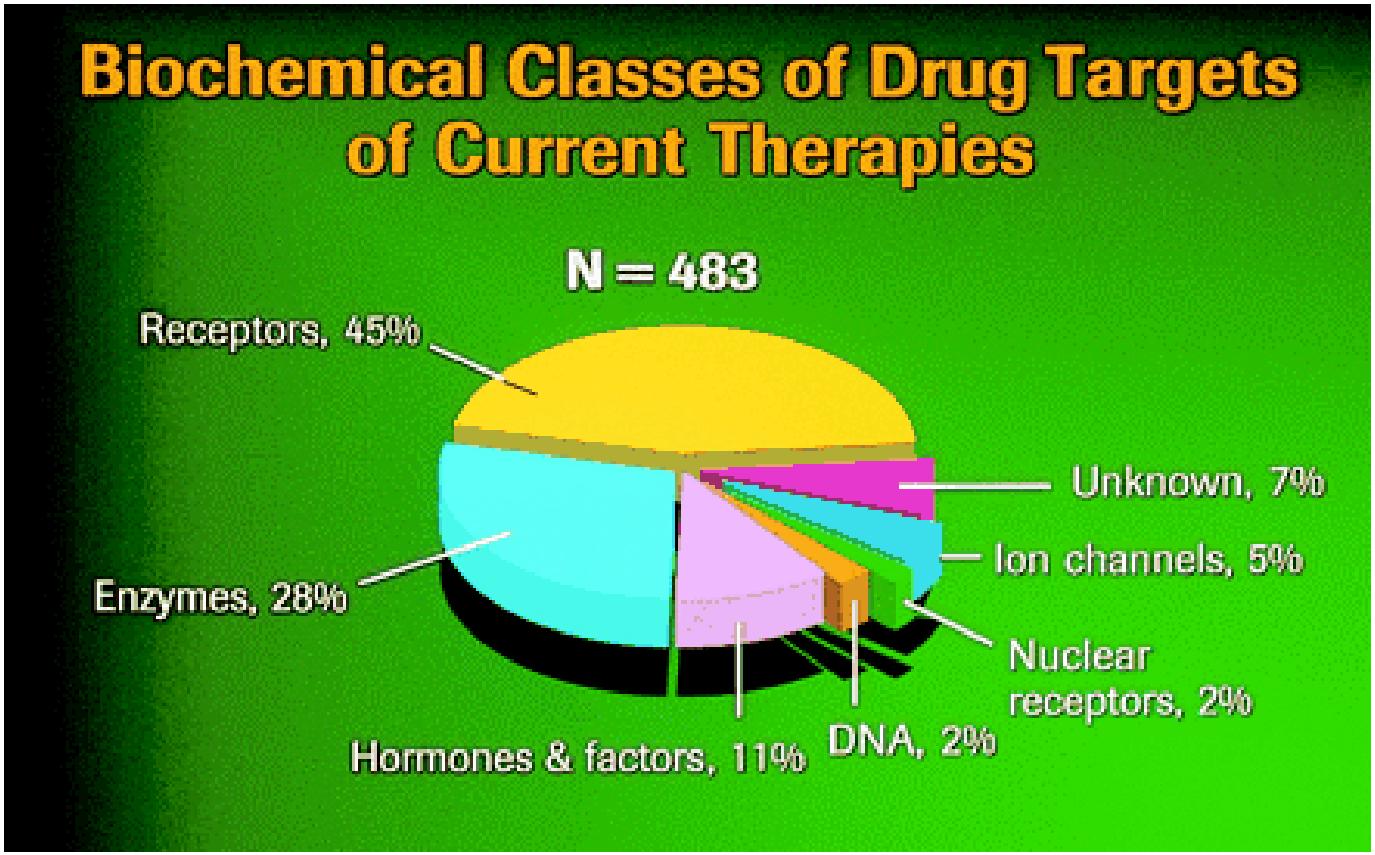
19,100 genes → ~1000 GPCRs → **~5% of genome**

HuGo (1999) → 100,000 genes → ~5000 GPCRs

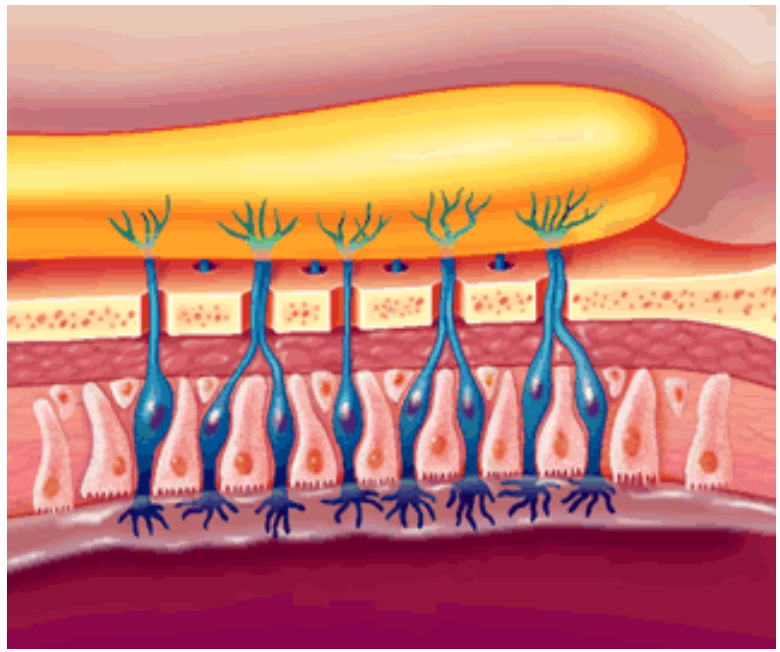
This calculation led to much excitement, speculation in pharmaceutical industry

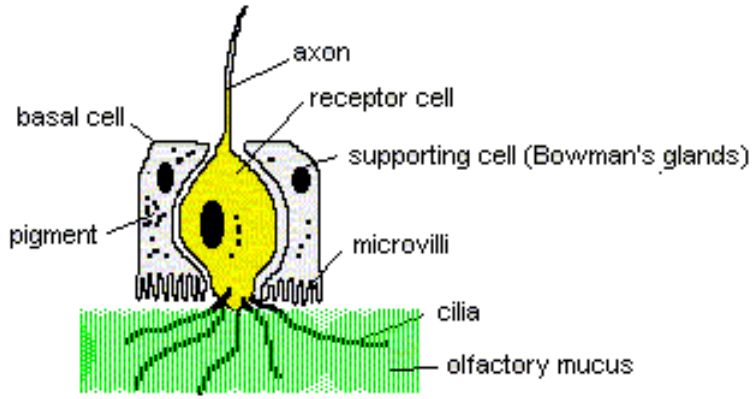
Orphan GPCRs are receptors whose ligand is unknown

In 2000 it was estimated that there are only about 500 drug targets for pharmaceuticals and most of those are receptors similar to GPC Receptors



But then there is the story of the nose





Nasal epithelium

Current estimates say that humans have roughly five million olfactory receptor cells, about as many as a mouse. Compare this to a bloodhound 100 million olfactory receptor cells.

Each nasal epithelium cell expresses on its surface a single GPC receptor.

Mouse, 1296 Olfactory Receptor genes of which about **1000** are functional. One in every 25 of the mouse's 30,000 genes-is connected to smell

Human 500 -1000 Olfactory Receptor genes. But 70% are nonfunctional, leaving **150 - 300** functional receptors.

Dolphins **0** functional Olfactory Receptor genes. Dolphins keep their blowholes sealed underwater and have turned their nasal cavities into sound generators.

Orphan GPCRs

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After correction for the size of the human genome and effect of the nose

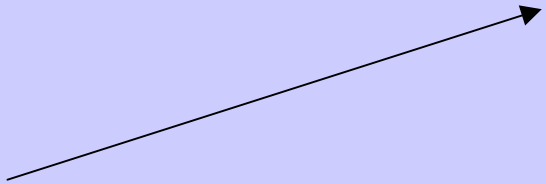
✓Current estimation ~**1000 GPCRs** in human genome

~700 olfactory, gustatory and chemokinine receptors

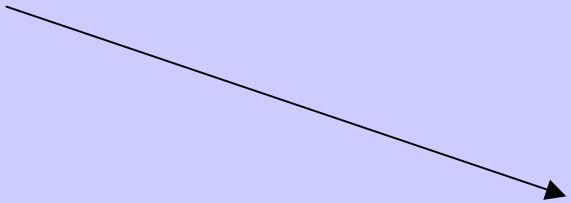
~300 transmitter GPCRs

Orphan GPCRs

160 GPCRs bind to 97 known ligands



~300 transmitter GPCRs



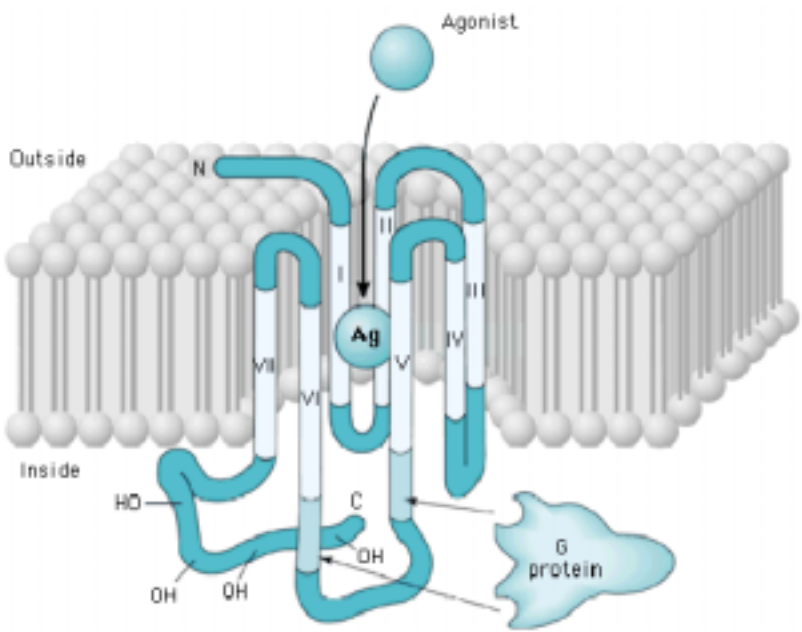
140 orphan GPCRs remain to be characterised

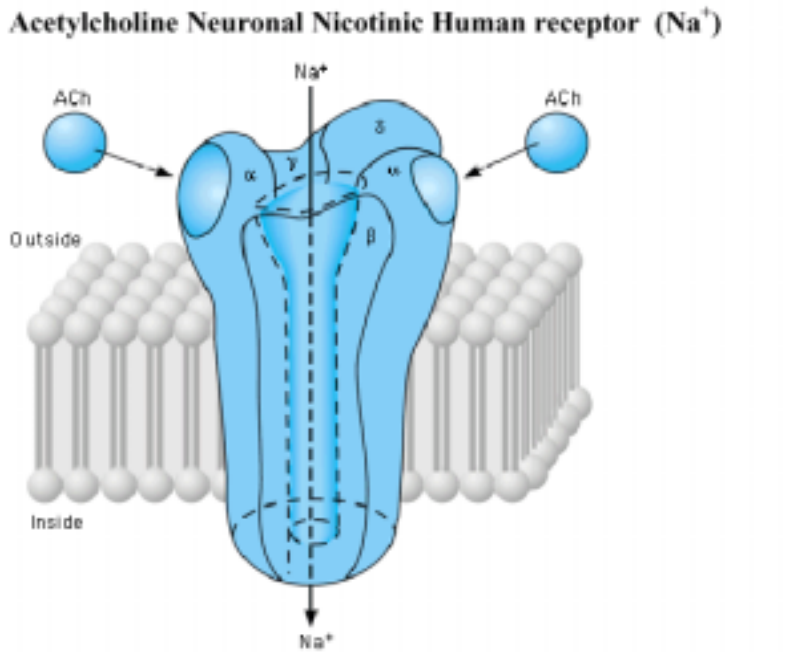
Would you bet your career on the existence of many new drug targets related to GPC Receptors ?

Would you bet your career on the existence of many new drug targets related to GPC Receptors ?

I vote No

Lessons from evolutionary biology and critical receptors





Natural Cholinergic Agonist and Antagonists

	Source of Compound	Mode of Action
Agonists		
Nicotine	Alkaloid prevalent in the tobacco plant	Activates nicotinic class of ACh receptors, locks the channel open
Muscarine	Alkaloid produced by *Amanita muscaria* mushrooms	Activates muscarinic class of ACh receptors
α-Latrotoxin	Protein produced by the black widow spider	Induces massive ACh release, possibly by acting as a Ca^{2+} ionophore
Antagonists		
Atropine (and related compound Scopolamine)	Alkaloid produced by the deadly nightshade, *Atropa belladonna*	Blocks ACh actions only at muscarinic receptors
Botulinus Toxin	Eight proteins produced by *Clostridium botulinum*	Inhibits the release of ACh
α-Bungarotoxin	Protein produced by *Bungarus* genus of snakes	Prevents ACh receptor channel opening
d-Tubocurarine	Active ingredient of curare from the bark of *Strychnos toxifera*	Prevents ACh receptor channel opening at motor end-plate

Lessons: **Trust Physiology**

Trust Evolutionary Biology

Keys to success in Bioinformatics