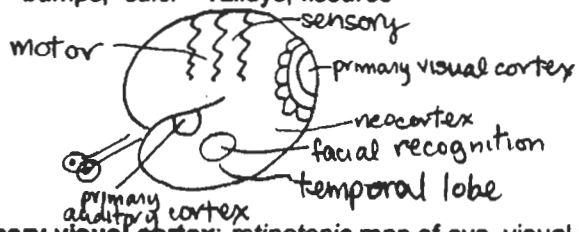
These are a set of notes from last year. (They won a $100.00 Neat Note Contest).
They are not completely infallible or comprehensive, but they are very good and should be helpful.
They won't work without class attendance!

Good Luck. The Management

2-06-02 Lecture 1 Introduction to the Nervous System

gyri – bumps; *sulci* – valleys, fissures



primary visual cortex: *retinotopic* map of eye, visual field

scotoma – contiguous visual blindness in one area of the visual field

- retinotopic maps: 1 primary map (V1), 1 secondary map, many subsidiary maps specialized (color, motion)
  - true for monkeys/people w/ appropriate visual stimulation during development

sensory cortex: projections from nerve endings in *somatotopic* organization

primary auditory cortex: pitch, location → map of auditory space

accessory areas: Wernicke (speech comprehension) damage → fluency, recognition nonsense
Broca (near motor area for mouth, lips; speech production) damage → slurred, slowed speech

H.M. – anterograde amnesia for declarative memories from bilateral hippocampal (+ accessory strucs) removal

mild temporal lobe epilepsy – personality changes rather than memory probs; Thom Jones, Fyodor Doestoevsky

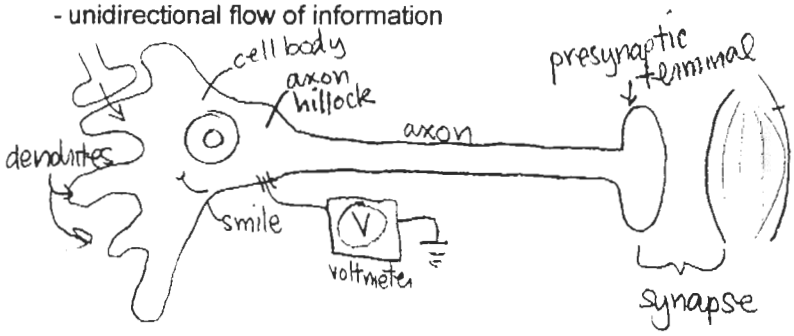
constellation of personality changes:
- hypergraphia
- hyper-religiosity
- hyposexual

Phineas Gage – frontal lobe damage. Bert → Cookie Monster.

frontal lobe – action planning, working memory, rational behavior

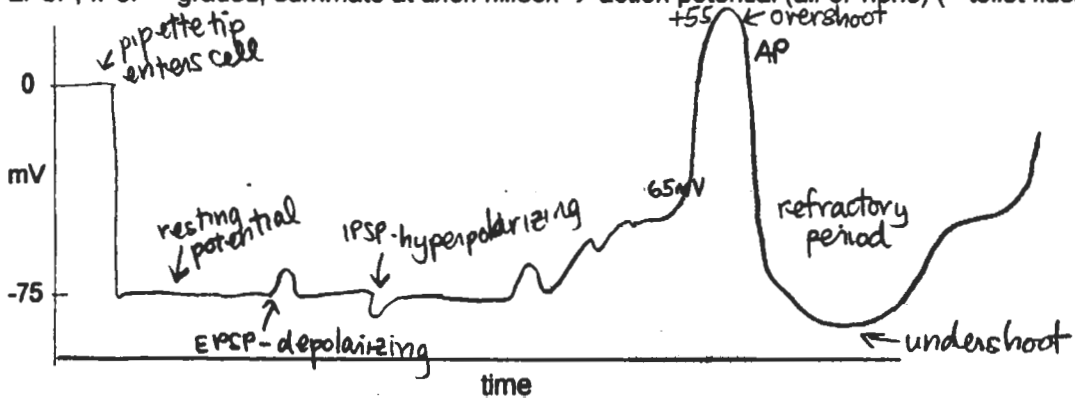
Fred the Neuron

- unidirectional flow of information



Cell recording: Synaptic potentials

EPSP, IPSP – graded, summate at *axon hillock* → action potential (all-or-none) (~ toilet flush)

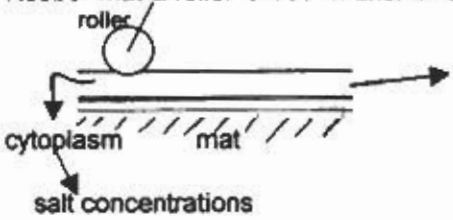


2-11-02 Lecture 2 Membrane Channels and Signaling

Action potential (AP) studied in giant squid axons (used for fast escape response) up to 1 mm in diameter

Hodgkin Baker Keynes Toothpaste Tube Experiment

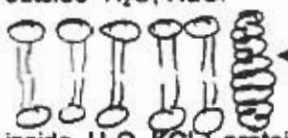
Rubber mat & roller → flatten axon like toothpaste tube → cytoplasm extruded



Reinflate w/ own salt soln → reproduced AP

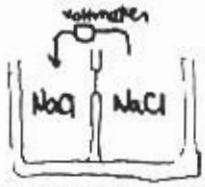
Singer-Nicholson Fluid Mosaic Model (of membrane)

outside H_2O, NaCl



phospholipid bilayer
← protein corks

electrical properties: resistance & capacitance
(from lipids & saltwater)



Make **black membrane** (artificial lipid bilayer)
- paint phospholipids over hole
- apply voltage → measure capacitance
- biological & artificial : **C = 1 μF/cm²** → C of biological membranes from lipids
- artificial: **R ≈ 10^{12} Ω•cm²** biological: **R ≈ 10^{6} - 10^{8} Ω•cm²** (10^{6}X more conductive) → R not just from lipids
- Conductance (g) / resistance (R) from proteins in membrane

capacitor – plates. **C = A / s**; A = area, s = plate separation

conductor	saltwater
insulator	lipid bilayer (dielectric constant = ~ olive oil)
conductor	saltwater

resistance – **R = V / I** (Ohm's law) = **ρl / A** (ρ = resistivity, = path length, A = cross-sectional area)
- in series: resistances add
- in parallel: conductances add; 1/R adds

conductance – **g = 1/R = I/V** [Siemens = $Ohms^{-1}$ = mhos]

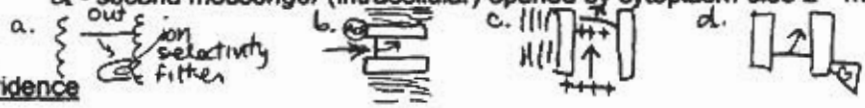
Conductance is engineered into biological membranes via protein **conductance channels** (doughnuts), which are:

a - *ion-selective*
- *gated*

b - *ligand* (extracellular; small molecules bound to outside) e.g. AchR opens in response to Ach binding

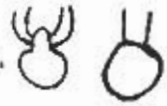
c - *voltage*: in squid axon, channels open in response to AP/membrane potentials. Membrane-spanning α-helices + lipid sidechains with every 3rd a.a. = Lys or Arg (charged)

d - *second messenger* (intracellular) opened by cytoplasm-side 2nd messenger



Evidence

- From **patch-clamping**: fire up micropipette tip so it's flat not sharp, stick against cell, not go inside; pull it up, usually makes a vesicle. Whole-cell recording.
  - Conductance fluctuates quantally in even multiples → open or closed, no partial states



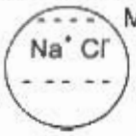
- From molecular bio & cloning:
  - Chinese krait snake (*Bungarus multicinctus*) – high affinity toxin that blocks AchR at neuromuscular junction, nearly irreversible binding → **α-bungarotoxin**. Snake → αBtx → digest & purify → AchR
  - Electric fish (rays & eels) – electric organs like DC batteries in series (50mV plates); electroplates: 1 side is all neuromuscular junction. Grind up fish → pure preparation of Ach (pure protein) → amino acid sequence
  - High-affinity toxin **tetradotoxin (TTX)** blocks Na^+ channels; Drosophila shaker mutant neuromusc jn lack K^+ ch
  - Use toxins to purify proteins → clone channels. Map mutation → clone DNA → look for channel seq homologs

2-13-02 Lecture 3 Ionic Basis of the Resting Potential

Sodium-Potassium Pumps

Want to engineer [ion] & conductances because:

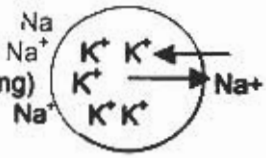
concentrated salt soln → H_2O flows down conc gradient into cell → lyse cell- *osmotic death* from McAnion burger



Makes ribozymes, protein; nucleic acids & proteins are negative → eat McAnion burger → net neg

So, **pump K^+ in, Na^+ out** using lots of ATP (main cost of brain).

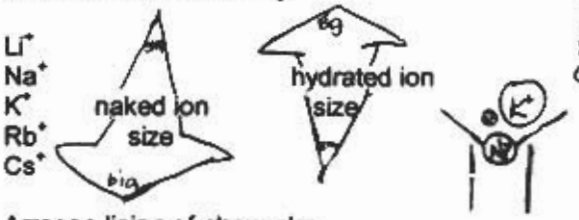
- Blocked by: *ouabain* (neurologists; quick to act, quick to wash out)
  digitalis (cardiologists use to make weak heart beat slower, strong)
- Blood [Na^+] ≈ saltwater [Na^+]



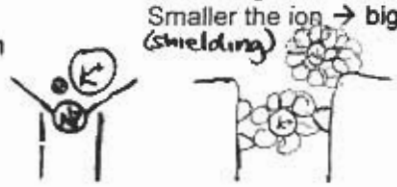
From toothpaste tube expt (Hodgkin):

- lots of Na^+ outside cell; K^+ rich, Na^+ poor inside cell.
- very little Cl^- inside due to McAnion burgers

Basis of Ion Selectivity



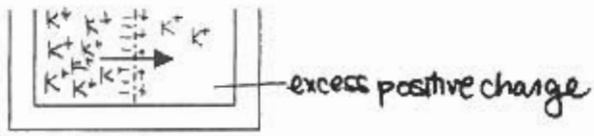
Ions in H_2O soln → shells of hydration.
Smaller the ion → bigger shell of hydration (shielding)



Arrange lining of channels:

- size hierarchy in Na^+ channels; let naked ions through → Li^+ should be even more conductive
- fill channel w/ water so hydrated ion goes through → Rb even more conductive
- K^+ channel has special lining of polar side chains so K^+ fits in pseudo-shell of hydration but Na^+ too small
- Engineer shell to fit only desired ion.

Predominant channel conductance is K^+.
Diffusion → net flow, excess positive charge → voltage gradient.
Chemical energy lost by going down conc. gradient.
Must be picked up going up voltage gradient.



Resting potential

$\Delta E_{conc} = \Delta E_{electric}$

$\Delta E_{conc} = RT \cdot \ln [K^+]_{concen.} - RT \cdot \ln [K^+]_{dilute} = RT \cdot \ln [K^+]_{concen} / [K^+]_{dilute}$

$\Delta E_{electric} = qV = zF \cdot V = RT \cdot \ln [K^+]_{out} / [K^+]_{in}$ z = charge # (1 for K^+, 2 for Ca^{+2})

Nernst Equation

$$V = \frac{RT}{zF} \ln \frac{[K^+]_o}{[K^+]_i}$$

With z = 1, T = 25°C, $V = 58mV \cdot \log_{10} [K^+]_{out} / [K^+]_{in}$

Resting potential $V = 58 \cdot \log [20]/[400] = 58 \cdot \log (1/20) = -58 \log 20 = -58 (1.2) =$ **- 69 mV**

→ most of the resting potential is from K^+ Nernst batteries

Bath-changing experiments

-Can change conc gradient, see how resting potential changes. $\Delta [K^+]_{out}$ by 10 → Δ log by 1 → $\Delta V = 58$

Almost entirely permeable to K^+, but nerve cells don't follow Nernst totally.

Goldman equation (Nernst eqn + fudge factors)

$$V = 58 \log \frac{[K^+]_o + P_{Na}/P_K [Na^+]_o + P_{Cl}/P_K [Cl^-]_i}{[K^+]_i + P_{Na}/P_K [Na^+]_i + P_{Cl}/P_K [Cl^-]_o}$$

P_x / P_y = permeability ratio = ability of membrane to allow something through the holes.
Permeability is a property of membranes.

If cell mostly permeable to Na^+: $V = 58 \log [440]/[50] = 58 \log 8.8 = +55$ mV (like top of AP)

AP like switching between 2 Nernst batteries/ permeabilities: -70 → +55 → -70 :: $E_K \rightarrow E_{Na} \rightarrow E_K$

E_K = Nernst equilibrium potential for K^+

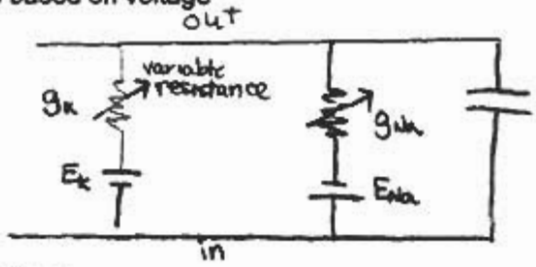
2-19-02 Lecture 4 Action Potential I

Hodkign & Huxley bath-changing experiments

- Change [Na^+] outside axon → top of AP changes
- What results in switching of ion conductance channels from K^+ to Na^+?
  - Gating mechanisms for K^+ v. Na^+ slightly different, though both voltage-gated
  - Defined threshold (60 mV) for axons → voltage important in determining AP

- Adrian & Hodgkin: axon + vasoline to block AP → absence of AP, still got some depolarization, not enough for AP → conducted voltage from AP → open & close channels based on voltage

$V = E_K = 58 \log [K^+]_o / [K^+]_i$
$I = V / R = g \cdot V$
$I_K = g_K (V_m - E_K)$
V_m = membrane voltage. When $V_m = E_K$, $I_K = 0$.



Ohm's Law for Membranes

$$I = I_K + I_{Na} = g_K (V_m - E_K) + g_{Na} (V_m - E_{Na})$$

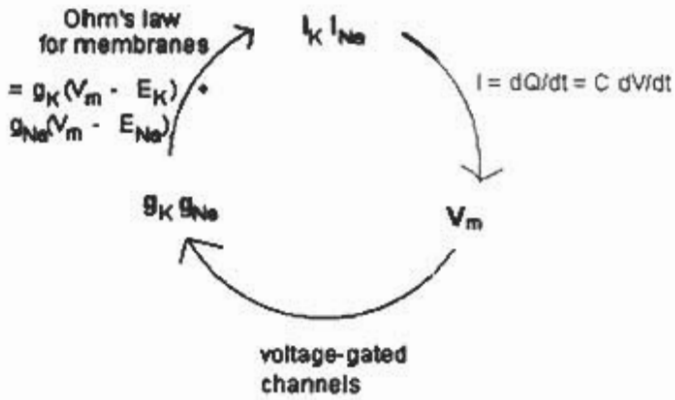
If simple Ohmic model is true & if g_K, g_{Na} are voltage-gated, then...

Hodgkin-Huxley predictive cycle

No spare charge in middle, charges all on surface → charging & discharging → capacitance

$Q = CV$ C doesn't change
$dQ/dt = I = C\ dV/dt + V\ dC/dt = C\ dV/dt$

$I = C \cdot dV/dt$

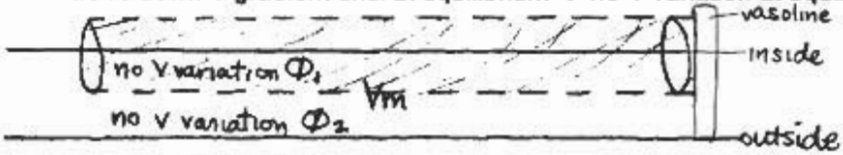


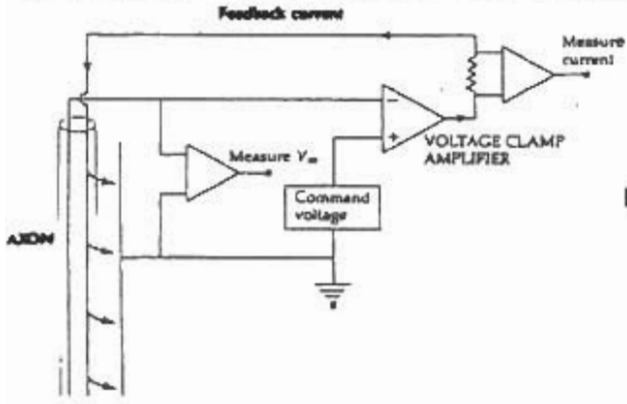
Hodkin & Huxley's Problems

- spatial variation in V_m
  - little patches of axon; all different → communicating
- temporal variation
  - can't hold conductance still to measure; axon V_m won't stay same
- How to separate I_K, I_{Na}

Solutions

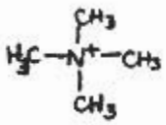
- *space clamp*: conductors through inside & outside so voltage/conductances are same for each patch of membrane; short circuit w/ wire threaded down middle of axon + vasoline so wires don't connect; I always flows down V gradient until at equilibrium → no V variation at equilibrium





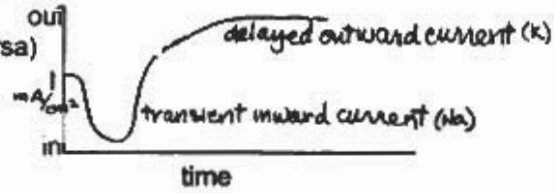
- *voltage clamp*: feedback system to wires (space clamp) to sense fluctuations in V → apply I to clamp at particular V to keep membrane at desired V. *differential amplifier*: input V → output I proportional to V. Impose command voltage. Measure feedback current. All current leaking out must be = I_{in} from voltage clamp amp.

- *bath-changing* (H&H) or *drugs* (modern method)
  - bath-changing: at +52 mV (~E_{Na}), no Na^+ current → pure K^+ current. Change E_{Na} by changing $[Na^+]_o$ → compare profiles
  - drugs: block channels. Block Na^+ w/ TTX → pure K^+ current (outward). Block K^+ with **tetraethylammonium (TEA)** → pure Na^+ currents (inward/transient)
  - Conductance profiles look like pure currents.
  - Voltage-dependent: ↑ depolarize → faster, greater conductance



Reversal potential: potential at which I flips from inward to outward (or vice versa)

- Negative current = inward (+ from outside to inside)
- I_{Na} should be inward, I_K should be outward (from voltage clamp trace)
- p. 99 handout



2-20-02 Lecture 5 Action Potential II

Depolarization opens Na^+ channels quickly. More I rushes in, ↑ depolarization, ↑ Na^+ conductance → positive feedback → explosive all-or-none AP driving membrane to E_{Na}

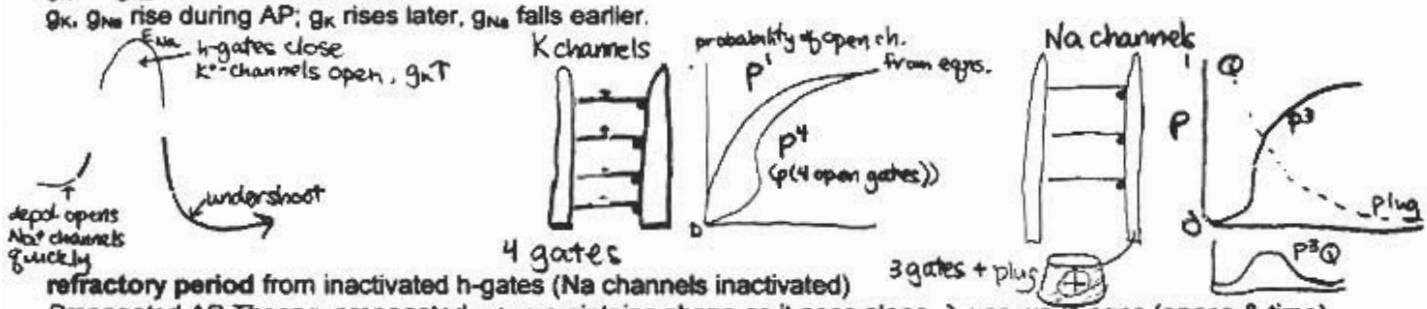
m-gate: rapid opening

h-gate/ inactivation gate: closes slowly after m-gate opens; can't be opened by ↑ depolarization

K^+ opens slowly in response to depolarization. Resting membrane is 100X more permeable to K^+ than Na^+.

$g_K >> g_{Na}$ at end → undershoot

g_K, g_{Na} rise during AP; g_K rises later, g_{Na} falls earlier.



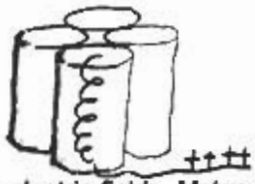
refractory period from inactivated h-gates (Na channels inactivated)

Propagated AP Theory: propagated wave maintains shape as it goes along → use wave eqns (space & time).

Mechanism of rise in g_{Na}, g_K is same but fall off is different. K gates fall from repolarization, Na falls while still depolarized → deliberately engineered to fall by h-gates, inactivation.

<u>K channels</u>: 4 α-helices w/ charged groups

<u>Na channels</u>: 4 pseudo-subunits



Voltages v. small but spread across Å → huge electric fields. Molecules can move from elec. field strength.

gating currents – charge movements/electrical events. Measure changes in charge distribution associated w/Na channel movement. Na channel opening with charged domains that move. Individual conductance channels are either all open or all closed, open probability predicted by g.

Eliminating inactivation: Na channels open but don't close. Depolarize w/o repolarize.
- pronase (proteolytic enzymes)
- DDT: abolishes inactivation of insect & crustacean axons.

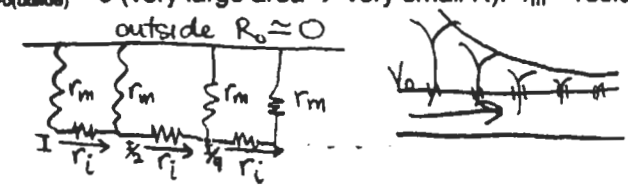
K channel w/ inactivation gate in fly contains cytoplasmic amino-terminal loop/tail w/ + charges.
Genetic deletion → no inactivation. Take away + charges → no inactivation. Inject peptides → restore inactivation.

<u>2-25-02 Lecture 6 Neurons as Conductors: Propagation of the Action Potential</u>

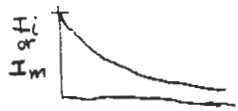
Permeability: property of membranes.
Conductance: property of system channels & ions
Harvard Bridge analogy: 3 am—permeable but not much traffic (conductance); rush hour → action potential
Resting potentials: use Goldman
Synaptic potentials, reversal potentials: use Ohm's law for membranes ($I = g_K(V_m - E_K) + g_{Na}(V_m - E_{Na})$)

Fred the neuron is almost the world's worst wire. Leakage → attenuation of signal.
Adrian & Huxley vasoline to block AP → found residual depolarization ahead of blocked AP (could contribute to AP)
→ some current spreads ahead of AP to depolarize the next patch of membrane

Patch of membrane ≈ **leaky cable** → leaky current flow, can be modeled by <u>ladder circuit</u>
electrotonic spread- passive spread of signals
$R_{o(outside)} \approx 0$ (very large area → very small R). r_m = resistance of membrane r_i = internal resistance.

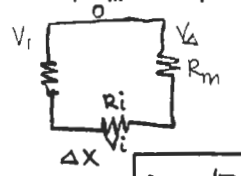


leaky water hose
pressure dissipates as go along, height dP/dx decreases w/ more holes



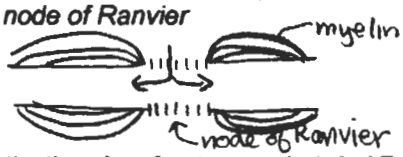
more holes, less insulation → lower r_m → more leakage → dV/dx greater (quicker V dissipates)
Skinnier hose → ↑ r_i → more water leakage. Better conductor, less holes → less attenuation.
r_i = core conductor. r_m = insulation, so attenuation goes with r_i / r_m → exponential. Attenuation ∝ leakage

1. $-dI_i/dx = I_m = V_m / R_m$, I_i = internal I, leaks
2. $-dV_m/dx = V_i = R_i I_i$ (Kirchoff's law)
3. $d^2V_m/dx^2 = R_i \cdot dI/dx = (1) = R_i V_m / R_m$



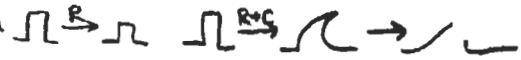
$V_m = e^{x/(\sqrt{Rm/Ri})}$ → scale factor, space constant/length constant $\lambda = \sqrt{R_m/R_i}$ $V(x) = V_0 e^{-x/\lambda}$

Current attenuation is a negative exponential space constant!
Longer space constant, slower decay → farther ahead you can depolarize the next patch over threshold.
speed of propagation ∝ λ → increase λ to increase speed
<u>Squid</u>: increased λ → minimize R_m, small R_i → giant axons
<u>People</u>: fewer holds all in one place → increase insulation w/ glial cells, *myelinated cells* → <u>saltatory conduction</u> through *node of Ranvier*

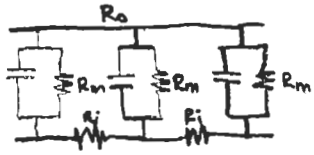


Na^+ inactivation → refractory period → AP doesn't spread in both directions
unidirectionality from fact that AP starts at hillock and travels to presynaptic end.

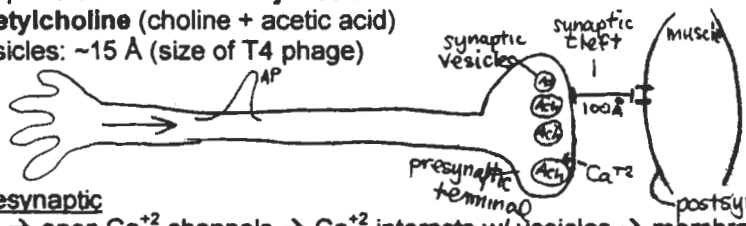
Membrane capacitance
- Doesn't matter for steady input, but does for brief pulses like AP or square wave
- For membranes (leaky cables) with R and C:
  - Fall-off of voltage signal is more pronounced than $e^{-x/\lambda}$
  - Signals are delayed and smeared out in time



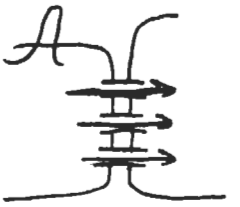
Giant axons and myelination → decreased C
Fewer Na^+ channels in cell bodies & dendrites → no AP



<u>2-27-02 Lecture 7 Electrical and Chemical Synaptic Transmission</u>
Chemical Synapses
simplest: **neuromuscular junction**: motor neuron → muscle
acetylcholine (choline + acetic acid)
vesicles: ~15 Å (size of T4 phage)



<u>Presynaptic</u>
AP → open Ca^{+2} channels → Ca^{+2} interacts w/ vesicles → membrane fusion → exocytosis → spew Ach into synaptic cleft
<u>Postsynaptic</u>: folds
Membranes w/ Ach receptors (ligand-gated) → bind Ach → open channels → let in current → depolarize → AP



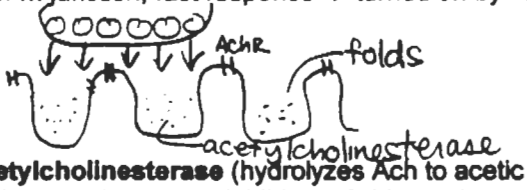
Electrical Synapses
- *gap junctions* – connections between pre and postsynaptic side; hexagonal hole for ions to go through
- quick & simple → pass on AP
- found in embryonic cells, crayfish (escape responses)

Postsynaptic Terminal

How do we know the synapse works?
- Otto Loewi. Frog hearts in separate dishes (1) —ringer soln→ (2)

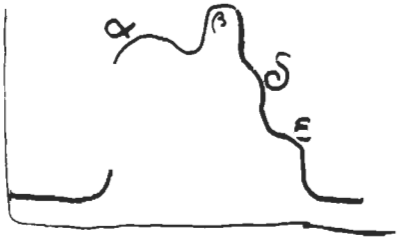
Stimulate vagus nerve → heart beats slower. Stimulate vagus nerve 1 → heart 2 beats slower. Substance in the ringer solution: vagusstoffe = acetylcholine.
Put Ach on neuromuscular junction → muscle contraction
Iontophoresis (like electrophoresis w/ions; spritz on ions) → gives tight spatial, temporal control of Ach application
Ach: mimics real neurotransmitter effects; abundant in ground up neuromuscular junctions
agonist – Ach, mimics biological response- could be the thing
antagonist – binds AchR but doesn't activate, like competitive inhibitors in substrate/enzyme binding
- succinyl choline, flaxedil (muscle relaxants, paralyze muscle)
- curare (β-D- tubocuranine; blow darts, v. effective)
- cobra toxin
- α-bungarotoxin (use tight binding to clone channels)

myasthenia gravis ("bad muscular weakness")- disease, make antibodies to own AchR. Use Achesterase inhibitors to allow Ach to stick around.
At n-m junction, fast response → turned on by flooding with Ach



acetylcholinesterase (hydrolyzes Ach to acetic acid + choline; use to turn off response)
spritz on Achesterase inhibitors → bigger, longer response
- neostigmine
- physostigmine
- Insectisides (Raid)
- Nerve gases (Sarin, Tabun, Vx)

How do we know Ach opens channels?
Record intracellularly from muscle, stimulate nerve; fire action potential & end-plate potential simultaneously.



α: compromise bt 2 events: AP + channels being driven to 0 mV
muscle: AP w/ own time constant
open channels getting driven to new potential (0 mV)

reversal potential: $I_{Na} = g_{Na} (V_m - E_{Na})$ → 0 when $V_m = E_{Na}$

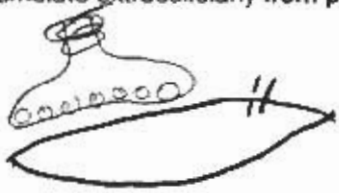
Muscle end-plate → Ach equally conductive to Na^+ & K^+
$I_o = g_{Na}E_{Na} + g_KE_K$. $g_{Na} = g_K$ → I_o = halfway between E_{Na}, E_K ≈ 15 mV (slightly more permeable to K^+ → 0 mV)
Add Ach to voltage clamped motor cell → individual openings → sign reversal @ 0 mV (in → out)

Ach Receptor
- 5 subunits. α subunit binds Ach (and α-bungarotoxin)
- α helices twist → open: K^+ out, Na^+ in → drive membrane to 0 mV

3-04-02 Lecture 8 Mechanisms of Transmitter Release at Synapses
How do you tell between an inhibitory and excitatory synapse?
Depolarizing ≠ excitatory.
If E is at threshold → neither inhibitory nor excitatory.

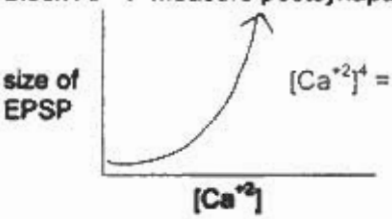
If reversal potential more:
- depolarized at threshold → excitatory
- hyperpolarized at threshold → inhibitory

Presynaptic Terminal
frog *sartorius* muscle
stimulate extracellularly from presynaptic motor nerve



record intracellularly from postsynaptic muscle (indirect measure)

Ca^{+2} is important! Enters through voltage-gated channel → induces vesicle fusion → quanta release
Series of experiments in low-Ca^{+2} solns.
Block AP → measure postsynaptic extrusion in different $[Ca^{+2}]$



- No Ca^{+2} in extracellular solution (used Co^{+2} antagonist in soln). Iontophoretically apply Ca^{+2}.
1. Neuromuscular transmission needs Ca^{+2}.
2. Spatial localization
   - needs Ca^{+2} near presynaptic terminal for normal transmission
3. Temporal localization
   - Ca^{+2} long before AP invasion → nothing
   - Ca^{+2} after AP → nothing
   - Ca^{+2} at AP invasion → little
   - Ca^{+2} just before AP → transmission
   - needs Ca^{+2} just before AP gets there

Leave nm junction alone (no extracellular stim) → mini potentials all same size & timing (quanta)
Katz: maybe quanta from vesicles releasing transmitter
Determine if mini-quanta are from Ach
- use Ach antagonists (e.g. curare) → bumps go away.
- use Achesterase inhibitors (e.g. neostygmine) → bumps get bigger, longer, potentiated

How to prove big neuromuscular EPSP is from little bumps?
- First, make AP smaller/disappear by lowering $[Ca^{+2}]$
- evoked release → small, spontaneous *miniature end-plate potentials* in multiples of 1 mV

Lots of vesicles waiting to be activated, but w/ low $[Ca^{+2}]$, small chance of activation.
Large synapse → independent probabilities; each vesicle has equal chance of release, can be described by:
Poisson distribution $P(x) = m^x e^{-m} / x!$, x = 0, 1, 2, 3... (# events)
m = mean quantal content, avg # (vesicles) released per stimulus; vary by varying $[Ca^{+2}]$

Frequency of failure = $\boxed{P(0) = e^{-m}}$ → if know frequency of failure, then know mean quantal content

Do quanta correspond to vesicle exocytosis?
- Arrange for AP to reach terminal just as piston goes down & smashes synapse onto frozen metal
  - Vesicles caught in the act of fusion
- Cell clamp mast cells w/ big vesicles, put AC current, measure impedance. current ∝ capacitance
  - capacitance increases stepwise manner ∝ surface area → area increasing from vesicle exocytosis
- Yes, secretion of quanta is result of vesicle exocytosis.

3-06-02 Lecture 9 Indirect Mechanisms of Synaptic Transmission

Quantal analysis

Presynaptic events affect probability of vesicle release → **m = mean quantal content** = avg # [vesicles] released per action potential, not amount of transmitter inside vesicles. All vesicles are created equal (for our purposes). measure Δ m → Δ p(vesicle release) → Δ presynaptic terminal

Postsynaptic events → Δ in mV response to transmitter = quantal size → Δ end-plate potentials
$\bar{v}_1$ = **quantal size** = postsynaptic response in mV to exocytosed vesicles worth of transmitter → change in postsynaptic cell or synaptic cleft

To find change in quantal content

- **Do something and wait for nothing to happen → look at frequency of failures, $P(0) = e^{-m}$**

To find change in quantal size

- **Do nothing and wait for something → measure response to nothing, spontaneous release**

Ca^{+2}-induced vesicle exocytosis

vesicle — Ca^{+2} — presynaptic membrane

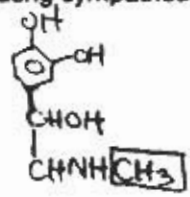
- Assay proteins at synapse: countable # of proteins in vesicle membranes
  - synaptotagmin (Zn^{+2} fingers)
  - synaptophysin
  - synaptobrevin (target for toxins)
  - rab (GTP-binding site) - GTPase
- Make clones of the proteins → gene sequencing
- Use toxins: **tetanus toxin** (blocks vesicle transmission at nm jxn)
  botulinium toxin (affect protein at presynaptic terminal)

Synaptic Modulation & Medium-complex Synaptic Behavior

Orbelli effect- potentiation of neuromuscular transmission when stimulating sympathetic nervous system

hormones/neurotransmitters:

- **adrenaline** (Latin) = **epinephrine** (Greek) = EPI
- **noradrenaline = norepinephrine** = NA → no methyl group



Fight or flight response → want to potentiate muscle
Spritz EPI or NA → potentiate nm jxns

To find out if presynaptic or postsynaptic

- Measure quantal size ($\bar{v}_1$) and quantal content (m)
  - Presynaptic (stronger synapse) → p(failure) decreases → increase m
  - Postsynaptic → increase in response → increase $\bar{v}_1$

→ shows both pre and post synaptic changes

NA binds to **α-adrenergic** receptors on presynaptic side → increase m
Epi binds to **β-adrenergic** receptors on postsynaptic side → increase $\bar{v}_1$

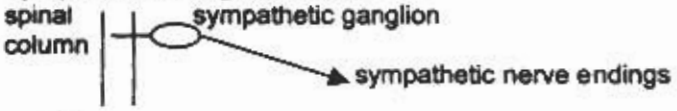
		Binds	Specific Agonist	Antagonist
Presynaptic	α-adrenergic r	NA	NA	clonidine

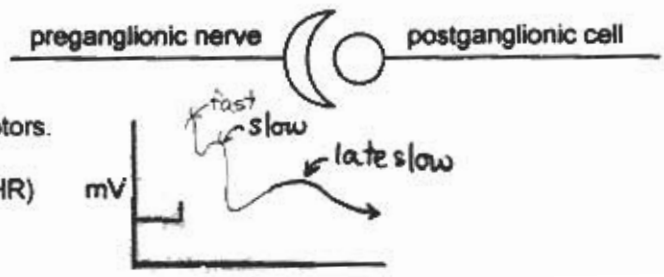
isoproterenol = used for anaphalactic shock
β-blockers – heart attack. stage fright

Pure α-activity: NA + β-propanolol → large increase in m (change in resistance, ingoing current same)
Pure β-activity: isoproterenol + clonidine → change in $\bar{v}_1$

3-11-02 Lecture 10 Biochemistry of Synaptic Transmission

Sympathetic Ganglia





excitatory transmission; 2 neurotransmitters to 3 kinds of receptors.
Stimulate preganglion, record from postganglion
Fast (nicotinic AchR), slow (muscarinic AchR), late slow (LHRHR)

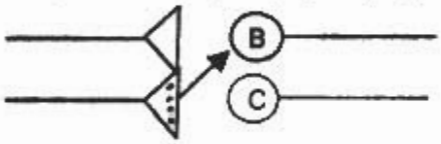
Response	Transmitter	Receptor
fast	Ach	nAchR (**nicotinic**)
slow	Ach	mAchR (**muscarinic**)
late slow	LHRH	LHRH receptor

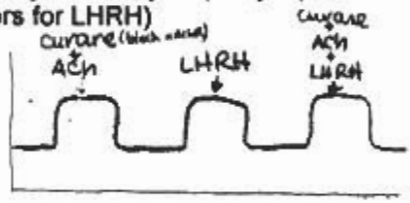
LHRH = luteinizing hormone releasing hormone
nAchR = ligand-gated channel to let in Na^+, ionotrophic
mAchR = looks like β-adrenergic receptor, rhodopsin: 7-member transmembrane, coupled to G-protein; close K^+ ch

Receptor	Agonist (potentiates effects)	Antagonist (blocks effects)
nAchR	nicotine	curare
mAchR	muscarine (from muscaria)	atropine (belladonna)

LH released from pituitary gland; 2 tissues- neuronal part sends signal to hormonal part.
iontophorese LHRH → late slow response

Late slow epsp only from B cells BUT immunoactivity stain only on presynapse of C cells → LHRH diffuses from C presynapse to B postsynapse (only B has receptors for LHRH)





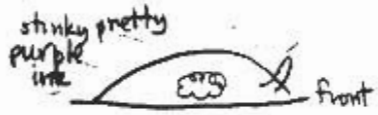
⇒ LHRH occludes mACH response

occlusion (cosaturation) experiments

- e.g. LHRH response occludes mAchR response
- implies converging downstream pathways
- LHRH & mAch → converge on closing m-channel using same 2nd messenger system

Aplysia californica

- learning-like effects
- traceable ganglionic circuit
- gill & siphon withdrawal response (reflex)
- monosynaptic component (sensory → motor) + polysynaptic (sensory → interneurons → motor)



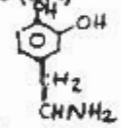
Reflex modulation

habituation- decrease in responsiveness to repeated stimulus (gill withdrawal reflex can habituate)
sensitization- increase in responsiveness following strong noxious stimulus; dishabituation (tail shock→strong withdrawal reflex)

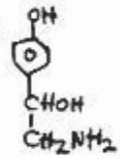
Presynaptic or postsynaptic- quantal analysis

- Stimulate sensory neuron extracellularly
- increase in rate of failures → presynaptic
- Post-tail shock, see: (monoamines)

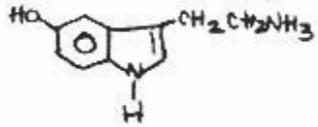
dopamine (DA)



octopamine



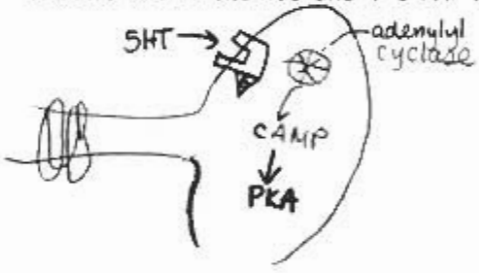
serotonin (5-HT, 5-hydroxytryptamine)

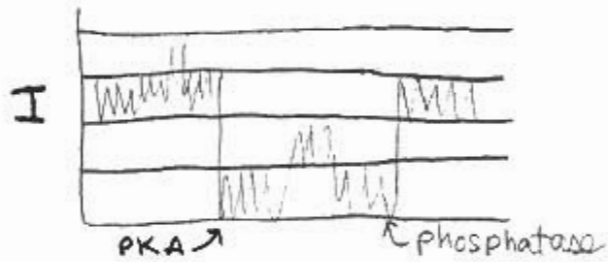


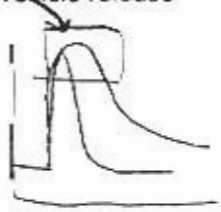
- increases in all 3 in abdominal ganglion after shock
- iontophorese them in area of synapse (sympathetic ganglion) → only 5-HT produces response
→ tail shock → 5-HT → increase cAMP (2nd messenger system)

PKA – catalytic (C) + regulatory (R) subunits; C phosphorylates proteins, R attaches to C to stop it; cAMP makes R drop off C to activate PKA
PKI (Walsh inhibitor) – constitutive inhibitor; shuts off PKA (binds to C, not to cAMP) → no transmission; blocks synaptic facilitation!

Apply 5-HT or tail shock to SN → repolarization down very short time later
Block K^+ channel (TEA) to make effect larger. Facilitation shuts off K^+ channel.
TEA + facilitation → bigger repolarization effect from decreased g_K
Close K^+ channel → delay repolarization of AP → more Ca^{+2} → more vesicle fusion → more transmitter release
Patch clamp expts → PKA closing K^+ channels (K-S channels)
Tail shock → excite neurons → 5-HT → cyclase → PKA → close K^+ channels → Ca^{+2} → more vesicle release







3-13-02 Lecture 11 Learning and Memory I

Identified cell co-culture experiment

- If you tie a string around axon so cell juice doesn't come out, culture SN & MN → they die
- Spritz 5-HT → synaptic facilitation in a dish for 1 hr
- 5 x 5-HT → facilitation for ≥ 24 hrs.
- + inhibitors of transcription (**actinomycinD**) & translation (**anisomycin**) → block short-term, not long-term facil.

Memory in a dish

1. *Morphological changes*
   - Aplysia: sensitized repeatedly → bigger & more synaptic (SN) endings
   - 4 proteins down regulated after LT facilitation (with 10 x 5-HT)

   ApCAM (Aplysia NCAM) – cell adhesion molecule, non-specific, abundantly made extracellular stickum downregulated → make less with neural plasticity → melt old connections

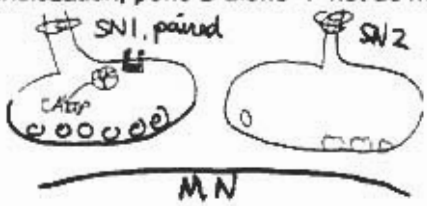
2. cAMP → PKA → K^+ S-channel closure; transcriptional events

CRE (cAMP-response element) - TGACGTCA → cAMP inducibility
ACTGCAGT

promoter bashing
CREB- PKA binding site for phosphorylation, binds to CRE
CREB* phosphorylation.
Inject CRE oligomers, but not other landing sites, into nucleus of SN → block LTM, not STM
Antibodies to CREB also block memory

Associative Learning

Pavlovian conditioning.
Dog: meat powder → salivation; bell → no saliva; meat powder + bell → saliva → bell → saliva
Can make Aplysia learning more specific.
Poke A + tail shock → poke A alone → jumpy, sensitization; poke B alone → not as much
Poke B + nothing



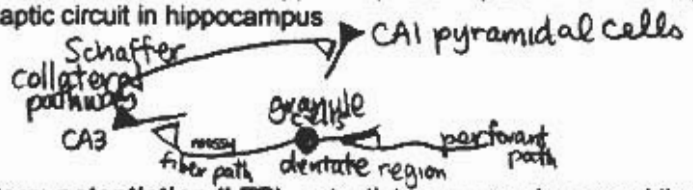
Pair aversive stimulus (or 5-HT) w/firing for one, not other. Both synapses stronger but paired → greater synaptic facilitation.

AP amplifies sensitization response.
In AP, Na^+ in, K^+ out, Ca^{+2} in. Ca^{+2} → 2nd msgr.
Train in Ca^{+2}–free soln → no amplification.
Ca^{+2} + 5-HT→ adenylyl cyclase → cAMP → learning
Ca^{+2} binds to calmodulin → CaM

Drosophila associative learning w/odors
Mutants that learn poorly:
- dunce: phosphodiesterase (PDE) problem
- rutabaga: adenylyl cyclase prob
- amnesiac: forgetful; mutation in structural gene for peptide neurotransmitter; bad CREB

Vertebrate Learning

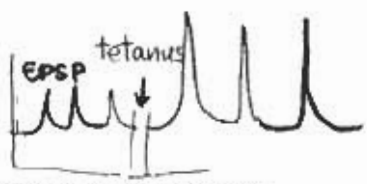
H.M. & ischemic patients → hippocampus is important for memory but memories aren't stored there
Trisynaptic circuit in hippocampus



long-term potentiation (LTP)- potentiate synapse, increased throughput
transmitter: glutamate (most excitatory synapses use glu)
Glu receptors in CA3 (metabotropic 7-transmembrane domain)

Agonist	AMPA	NMDA
Antagonist	CNQX	APV

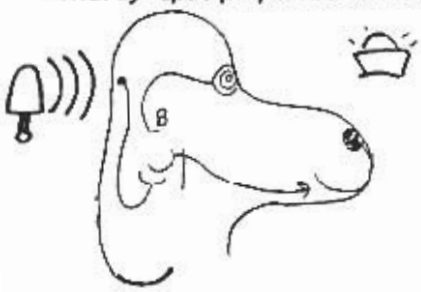
specific to receptor subtypes
block AMPA → block everything. block NMDA → block plasticity



3-18-02 Lecture 12 Learning and Memory II

D.O. Hebb

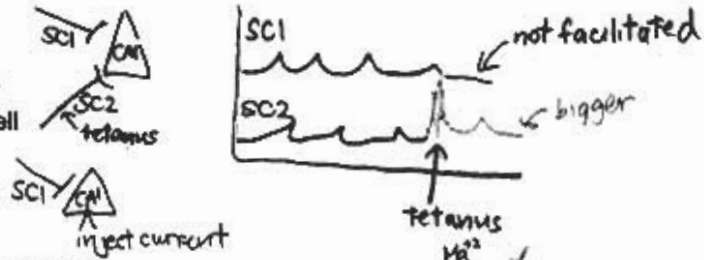
What synaptic properties are needed for animals to learn associatively?



- Synaptic path from visual stimulus to salivation
- Synapse BA is invariant, excitatory (BORING)
- Latent pathway CA in existence
  - Can teach dog in 15-30 min; faster than able to make new synapses
- Synapse CA initially ineffective (ring bell→nothing), but strengthened if C + A fire simultaneously → **Hebbian synapse**
- Presynaptic terminal releases transmitter at same time as postsynaptic cell fires (depolarization)
- Hebbian synapses found in CA1 cells

Is LTP Hebbian?

- Tetanus specific to synapse
- Single pulse SC_1 + postsynaptic depolarization (current injection) → LTP
- If tetanus to SC_1 + hyperpolarize CA1 cell (negative current injection) → no LTP
- LTP appears Hebbian



NMDA Receptors

- ligand-gated → opened by glutamate (or NMDA)
- Mg^{+2} blocks channel from ion flow
  - postsynaptic depolarization → charge repulsion → kick out Mg^{+2}
- permeable to Na^+ and Ca^{+2} (2^{nd} messenger)
- voltage and ligand-gated → *simultaneity detector*

AMPA Receptors

- permeable to Na^+

Is Ca^{+2} important for LTP?

- Yes!
- Inject Ca^{+2} into CA1 → LTP
  - Ca^{+2} can substitute for simultaneous firing
- What does Ca^{+2} do?
  - 2^{nd} messenger effects of Ca^{+2}
    - binds to *calmodulin* → changes shape → CaM adaptor → activates CaM kinase II
    - activates *protein kinase C*

Kinases

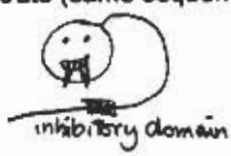
phosphorylates things w/ catalytic subunit

contains inhibitory domain saying "Ooh, phosphorylate *me*!"- pseudosubstrate (same sequence as P-sites on substrate)

CaM kinase II blocks pseudosubstrate sites → free up catalytic subunits

- block CaM kinase II → no LTP

PKC induces a shape change → free up catalytic subunits



Both pre- and post- synaptic effects found.

Experiments

H.M. → no declarative memory

Morris water maze task

- kiddie pool w/no visual cues, filled w/ milky water; only spatial cues on walls- distal cues; hidden platform
- spatial learning task
- Beandry, Lynch & Morris: shoot up rats intraventricularly w/APV →blocks LTP→blocks water maze learning
- Tonegawa: knockout NMDA receptors in CA1 cells → no LTP