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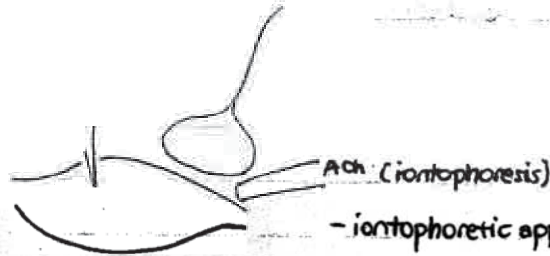
3/1/04

- neuromuscular junction is net excitatory AChRs let in Na^+ + K^+, but net effect from Na^+ influx)

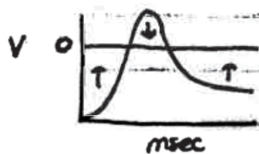


- iontophoretic application of ACh mimics presynaptic stimulation

- antagonists match concentrations of inhibition between presynaptic + iontophoretic ACh application

Katz et al - used curare to diminish EPSP below threshold, get normal peak (in case w/o curare, enough depolarization to cause action potential)
- either Na^+ or Ca^{2+} will give you depolarizing current; in this case, is Na^+

- synaptic potential depolarizes membrane if $V_m < 0$ mV; hyperpolarizes if $V_m > 0$ mV



- in voltage-clamp experiments, reversal potential of Na^+ is where current turned from depolarizing to hyperpolarizing (b/c $I_{Na} = g_{Na}(V_m - E_{Na})$)
  - ↳ if greater or less than E_{Na}, makes sign difference for I_{Na}

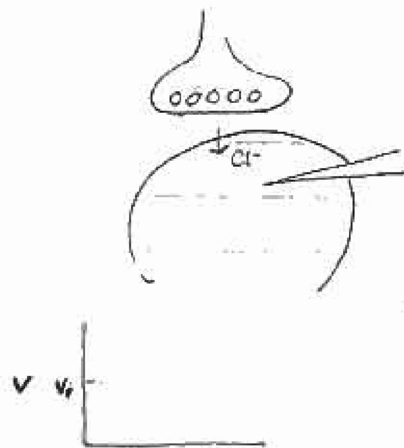
- w/ patch clamp:
  - give neuromuscular junction collagenase to remove presynaptic component
  - take patch of postsynaptic membrane where synapse used to be (w/ 1 AChR, or just a few)
  - voltage clamp patch, look for currents (none around 0mV)
    - reversal potential of synaptic channels ~ 0 mV
      - b/c AChR has huge pore, conducts Na^+ and K^+, → compromise voltage

at reversal potential, I = 0 (open channel = no net current)
- $I = 0 = g_{Na}(V_m - E_{Na}) + g_K(V_m - E_K)$
  - 0 −80 0 +55 get conductance ratio of g_{Na}/g_K (almost equal)

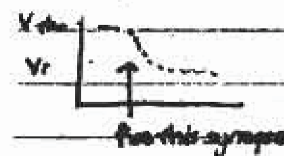
- if g_{Na} + g_K were equal, reversal potential would be ~ -15 mV
- if no other channels, just AChRs, reversal potential ~ 0 mV

- big depolarization only under nerve terminal
  - if below threshold, get exponential decay w/ distance from postsynaptic site (passive spread)

- in brain, other ligand-gated channels that gate other ions (eg. Cl^-, or pure K^+)
  - if only K^+ ions conducted, reversal potential for that channel + that synapse = E_K
    - will be inhibitory (?)
  - Cl^- ions not active pumped, arrange gradient passively, E_{Cl} will be V_m (~ -70 mV)
    - if open Cl^- channels, will drive membrane towards resting potential



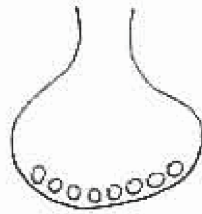
at resting, V_m already = Cl^- (E_{Cl})
get neither depolarization nor hyperpolarization
"silent synapse" at resting
- inhibitory b/c stabilizes voltage, makes harder to depolarize
- if other ions has depolarized almost to threshold, this synapse will drive V_m back towards resting, away from threshold



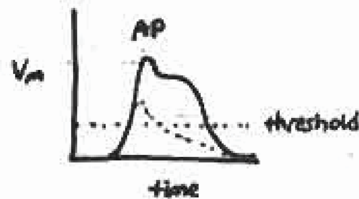
- if have synapse that lets in Na^+ + Cl^-, will depolarize membrane to mV below threshold, will not fire AP : w/ other synapses, still will not get AP
  - if reversal potential of synapse at threshold, neither inhibitory or excitatory
    - if $E_{reversal}$ below, will be depolarizing inhibitory

- if synapse opens ion channels, will have reversal potential
  - other synaptic channels let in other ions; even if depolarize a little, still inhibitory if drive V_m below threshold, make APs less likely

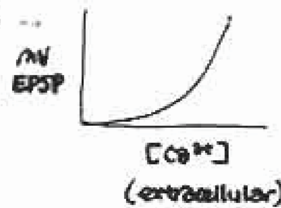
Presynaptic Side:



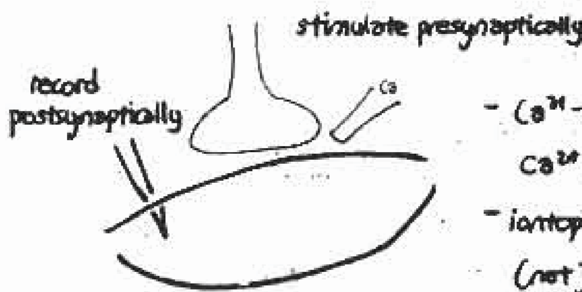
- Ca^{2+} comes in through voltage-gated Ca^{2+} channels and induces vesicle exocytosis
  - evo evidence all from Katz, frog sartorius neuromuscular junction
  - whole AP complicated, difficult to mess with



- if remove Ca^{2+} from bath, doesn't do much to AP, but makes synaptic signaling go away
  - at very low $[Ca^{2+}]$, get:



(on log plot, get straight line w/ slope of 4: takes 4 Ca^{2+} to get your reaction)
↳ exocytosis



[just enough to settle, not block all Ca^{2+}

- Ca^{2+}-free bath (actually added Co^{2+}, blocker of Ca^{2+} channels; antagonist) ← b/c damaged cells give off Ca^{2+}
- iontophoresed Ca^{2+} into vicinity of presynaptic terminal (not just anywhere, so not general health of cell)
  - w/ iontophoresis, control both place + time of injection
    - if apply right after AP, no response
      long time before AP, no response
    - must apply <u>right before</u> AP gets to terminal

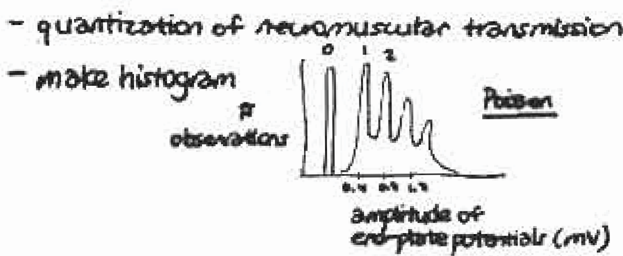
(Co^{2+} binding reversible: if $[Ca^{2+}]$ very high, will let Ca^{2+} win kinetic race by mass action)

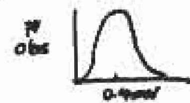
what about AP lets Ca^{2+} in? just voltage?
- blocked Na^+ channels w/ TTX, injected current so passive spread of current depolarizes
  ↳ no AP

- therefore, b/c works just the same w/ artificial depolarization, Ca^{2+} must be going through voltage-gated channels
  - Ca^{2+} must be outside terminal just when terminal depolarized
  - injecting Ca^{2+} into presynaptic side of squid giant synapse gives same response

- miniature synaptic potentials from prep where Katz didn't do anything (no stimulation): got tiny, 1 mV deflections spontaneously
  - from release of single quanta of ACh spontaneously
- found out mEPSPs blocked by curare (blocked by half at ~~cura~~ curare blocking 50% AChR), adding eserine (AChE inhibitor) makes minis bigger + longer
  - this is how Katz proved minis from ACh
- normal neuromuscular transmission made up of these bumps? normally can't find out b/c of 30 mV change even w/ decreased end-plate potential: will never see single quanta
  - must make transmission less efficient: decrease Ca^{2+} (extracellular in bath)
  - this way, get transmission w/ quanta in 1's, 2's, 3's

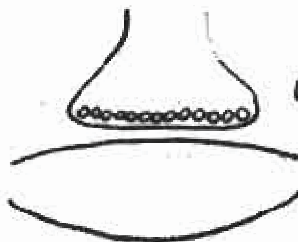
- same neuromuscular transmission, but less efficient, w/ less Ca^{2+}
  - do over, get bump, nothing, bump, have to do experiment over and over (~50% failures)
  - however, bumps don't come in arbitrary sizes: come in 0, 1, 2, sometimes 3
    - quantization of neuromuscular transmission
    - make histogram





- noise, but Gaussian distribution around 0.4 mV (amplitude of spontaneous mEPSPs)

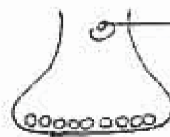
  - evoked transmission also quantized



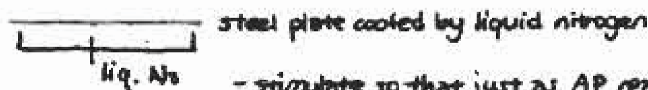
LOTS of vesicles, each w/ infinitesmally small chance of fusing (but each w/ same chance)

- Poisson distribution
  average # vesicles released per stimulus = mean quantal content (m)
- predict # of failures $P_x = (m^x e^{-m}) / x!$ probability of having X vesicles released

- if assume lots of vesicles, all equal, independent release, can predict Poisson distribution and this fits the data (tells us about vesicle kinetics)
  - so far, we don't know that quanta are vesicles
  - doing EM shows you vesicles full of transmitter
- evidence for vesicle exocytosis (2):

1. 

EM doesn't show you vesicle exocytosis (too fast to fix)



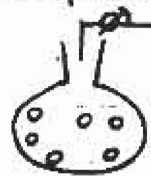
- stimulate so that just as AP reaches terminal, piston falls on plate

- then, take freeze-fracture slides

all ice: get cleavage between layers of lipid membrane: shade w/ osmium, take EM

- if stimulate exactly when freezing, get bumps (vesicles exocytosing)
  - quanta correspond to vesicle exocytosis, it seems

2. patch clamp, apply suction, continuous recording between inside of cell & electrode
   ↳ take out patch so recording into cell
   - high frequency stimulation : measure capacitance (larger cell = more current?)
     - measure stepwise increases in capacitance (stepwise increases in membrane surface area from membrane fusion)



⇒

$C \propto \frac{A}{d}$

- how does Ca^{2+} cause exocytosis?
  - proteins in synaptic vesicles are countable number; targets for toxins
    - botox, eg: permanent blocker of transmission at neuromuscular junctions (very potent toxin, only 1000 molecules to kill mouse)

tetanus toxin also paralyzes neuromuscular junction

- these 2 toxins both proteases that cleave synaptobrevin (disrupt vesicle fusion, neuromuscular transmission)

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