- collagenase to get postsynaptic side clear, patch clamp (w/ ACh in blunt microelectrode), look at current flow (magnitude & direction)  
  - voltage clamp  
  (\( V_m < 0 \), current negative = inward)  
  (\( V_m > 0 \), current negative = outward)

- ionotropic receptors have reversal potentials:  
  - if \( E_{\text{reversal}} \) above or below threshold, determines if excitatory or inhibitory  
  - if \( E_{\text{reversal}} = \) threshold, will not affect either way

- stimulate extracellularly presynaptic, record intracellularly postsynaptic  
  - need Ca\(^{2+}\) (\( \text{Ca}^{2+} \)) for presynaptic transmitter release, EPSP  
  - cobalt interferes w/ natural Ca\(^{2+}\) leak from slightly damaged muscle

  \[
  \text{Ca}^{2+} \text{ must be near synaptic cleft, present around when AP reaches terminal (or right before reaches terminal.)}
  \]

  treated whole system w/ TTX, increased stimulus intensity to passively conduct to terminal:  
  found that pure voltage could mimic AP: evidence for voltage-gated Ca\(^{2+}\) channels  
  - intracellular presynaptic injection of Ca\(^{2+}\) also gives EPSP

  - minis - correspond to small releases of ACh (b/c can affect w/ curare, neostigmine)  
    - make up m\& EPSP: reduce Ca\(^{2+}\), stimulate presynaptically, record postsynaptically  
      - effect comes in quantaal sizes (\( M_i \))
    - Gaussian distribution around \( 0.4 \text{mV} \)  
      - direct evidence for quantaal transmission  
    - large number of vesicles w/ equal \( p_i \), independent probability of release (from Poisson curve assumptions)
- make preparations of synapses, synaptic vesicles
  - synaptobrevin - V-SNAREs (important in exocytosis)
    - target of botulinum toxin (BOTOX) & tetanus toxin
    - mutants homologous to yeast mutants w/ exocytotic defects
    - yeast cycle vesicles between inner & outer lamellae
    - bad cycling due to mutant v-snares & t-snares
    - vesicle endocytosis looked at in cell-free dog pancreas system
      - same proteins showed up
  - synaptotagmin - Ca\textsuperscript{2+} binding domain: this is the calcium sensor for vesicle exocytosis

- neuromuscular junctions can be modulated
  (eg by chip screening - potentiated by adrenalin
  - potentiated by sympathetic stimulation (neuromuscular transmission)
    - Orbelli effect (controversy: presynaptic or postsynaptic?): turns out is both
      - do quantal analysis before + after sympathetic stimulation: more quantas released
        per action potential will give more double, triple, etc releases (average # released),
        if presynaptic effect; if postsynaptic (eg more ACh Rs, or more sensitive ACh Rs,
        will not affect placement of peaks by shifting histogram right (eg 0.4 mV \rightarrow 0.6 mV)
        - \( P_w = e^{-m} \frac{m^2}{x!} \) (but there is better way)

To measure change in quantal size, look at minis, look at size of mEPSPs
- this will increase if Orbelli effect postsynaptic
- quantal size \( \bar{V}_q = \text{average voltage displacement of quantum of spontaneous miniature potential} \)
  \( \neq \text{physical size of vesicle (all same size, same density of transmitter)} \)
norepinephrine

Orbelli effect: more adrenaline comes down sympathetic axons to neuromuscular junction

Adrenal gland secretes another compound (cylinder around your body): norepinephrine

→ there result in presynaptic enhanced quanta release (NE)

→ also postsynaptic response from circulating hormone (EPI)

phenylalanine → tyrosine → tyramine → dopamine → norepinephrine

adrenaline ← (norepinephrine) → NE

adrenaline from adrenal medulla (on top of kidney)

epinephrine same in Greek

4 EPI

norepinephrine + epinephrine both from phenylalanine, differ by methyl group

β-propanol

antagonist: isoproterenol (EPI antagonist)

drug used for asthma in hospitals, stage fright

→ applying this gives no change in quanta size

→ better music performance.

epinephrine → agonist = isoproterenol

(adrenaline) antagonist = β-propanol (blocks adrenergic response on postsynaptic side)

presynaptic: look at number of failures (Cuba found less failures)

norepinephrine → agonist = norepinephrine

(norepinephrine) antagonist = clonidine (blocks presynaptic response, gives pure postsynaptic)

postsynaptic: look at quanta size (will be bigger w/ Orbelli-effect)

→ postsynaptic so increase actually from epinephrine closing leakage channels

depolarizing current leaks out less (Ringel)

V = IR

so RT, VT

depolarizes more & longer

I from ionicotropic AChRs: doesn't change, depolarizes, but leak out big muscle is big: plug up leakage holes in muscle, increase R
- modulated synapses - have volume control
- norepinephrine + epinephrine are modulatory transmitters (gain control)