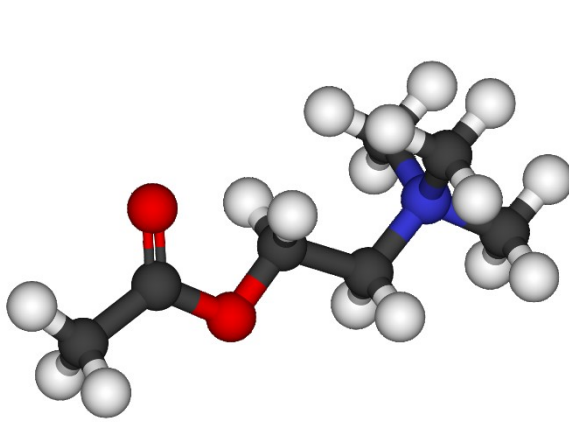
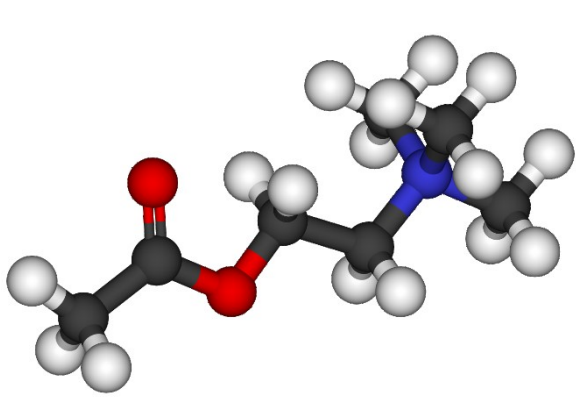
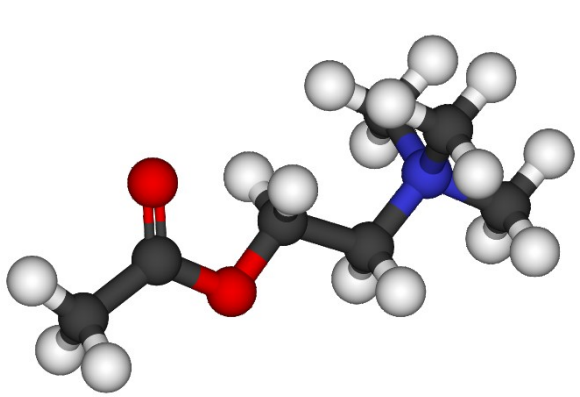
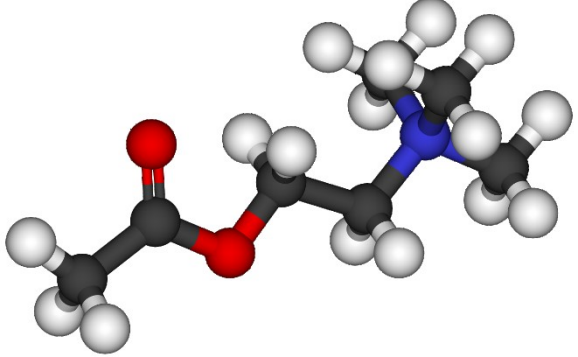

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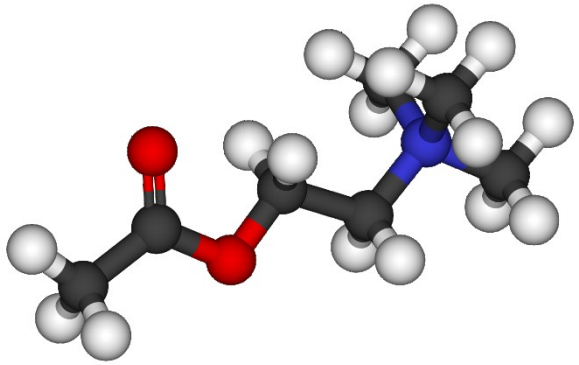
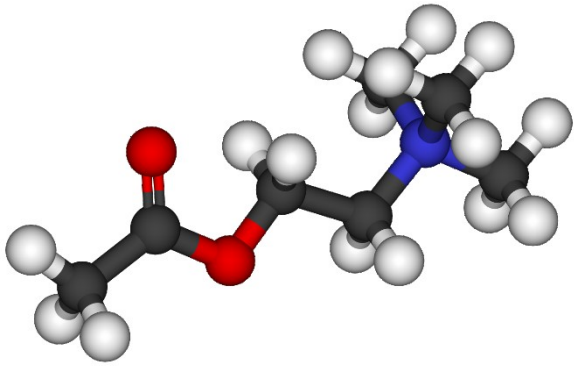

THE STATISTICAL NATURE OF THE ACETYCHOLINE POTENTIAL AND ITS MOLECULAR COMPONENTS

BY B. KATZ AND R. MILEDI

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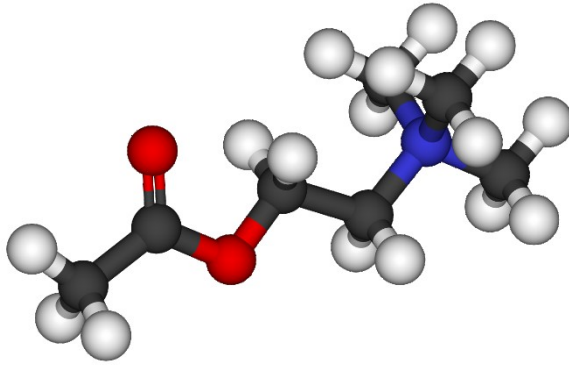
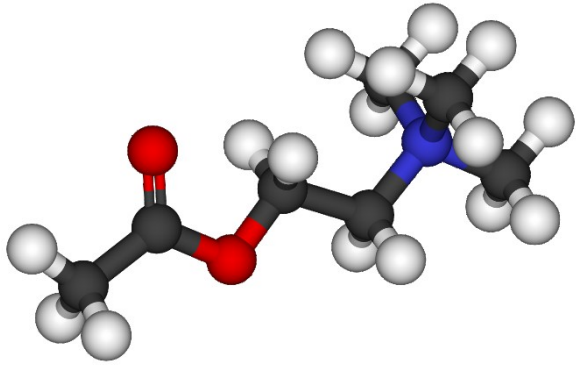
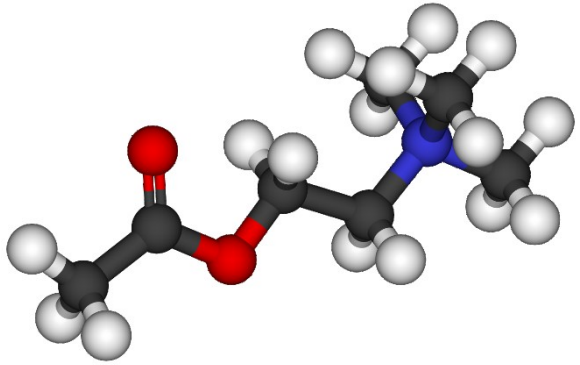
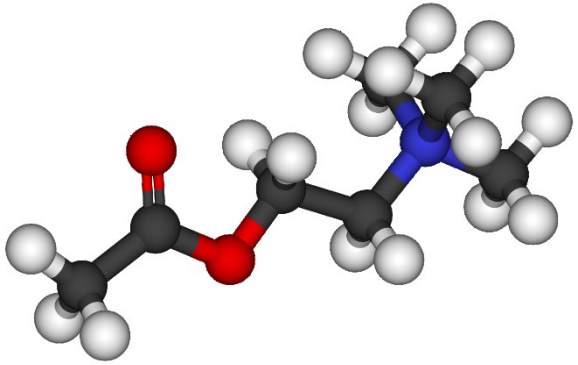





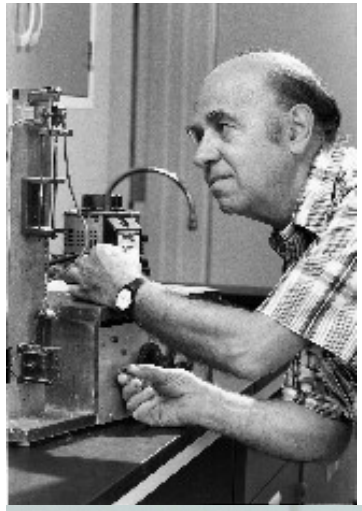
Bernard Katz (1911-2003)

- German-born biophysicist and neurophysiologist.
- Studied medicine at the University of Leipzig, then fled to Britain in 1935.
- Worked at University College London (UCL) under Archibald Hill (Nobel Laureate 1922).
- Finished PhD in 1938, then moved to the University of Sydney.
- Worked with John Eccles (Nobel Laureate 1963 with H&H).
- Radar officer for the RAAF during WWII.
- Made a professor at UCL in 1952.

Notables:

- Fellow of the Royal Society
- Knighted in **1970**
- Nobel Prize in Physiology or Medicine in **1970** (with Julius Axelrod and Ulf von Euler).

Ricardo Miledi (1927-)



- Mexican-born neurophysiologist.
- Was educated and worked in Mexico until moving to UCL in 1958.
- Distinguished professor at UC-Irvine since 1984.
- Roughly 500 publications and counting (according to his CV).

- You have to scroll to see all his honors and awards…

Notables:

- Member of the National Academy of Sciences (USA) – 1989
- Member of the Mexican Academy of Sciences – 1991
- Royal Medal of the Royal Society, England – 1998
- Society for Neuroscience's Ralph W. Gerard Prize - 2010

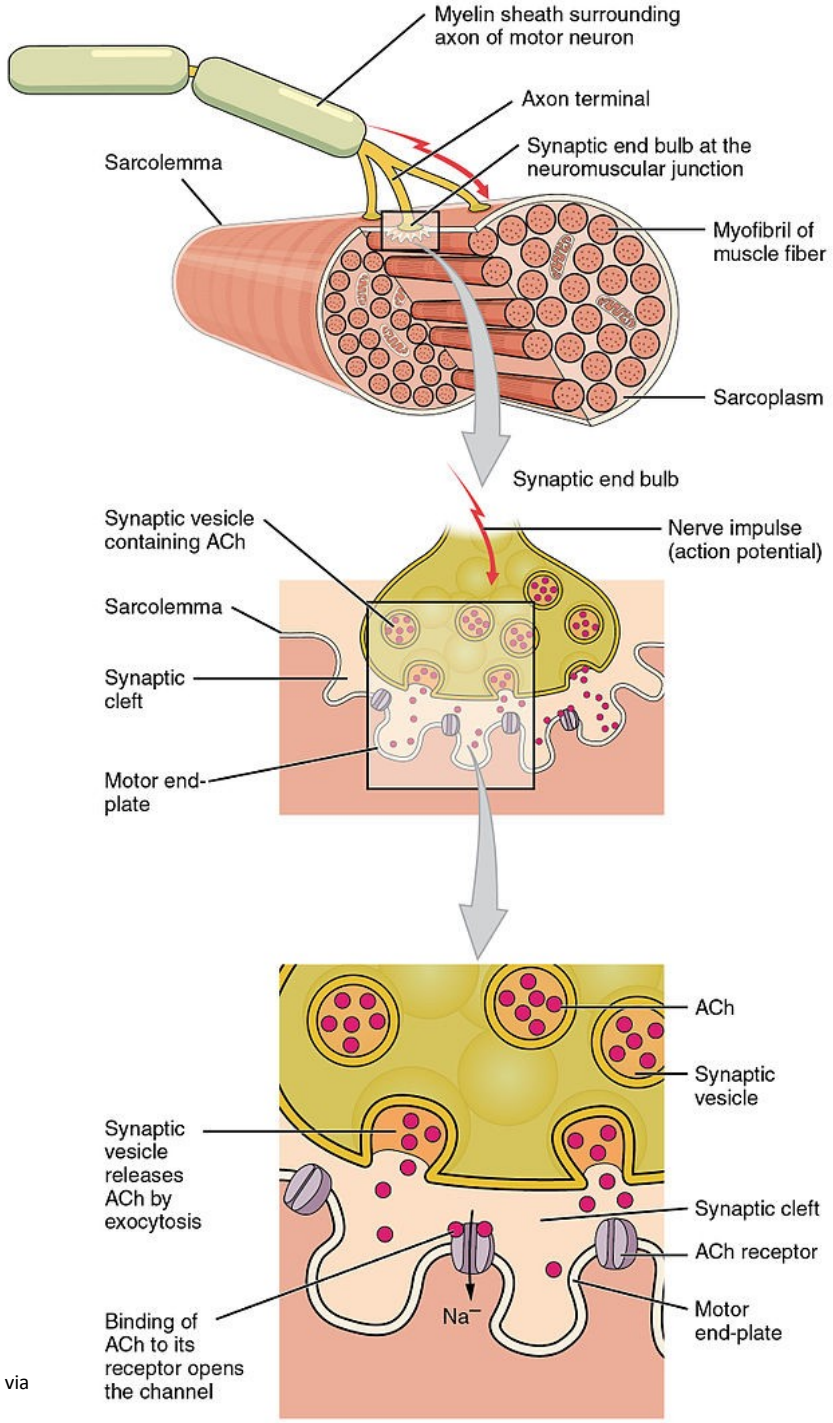
Acetylcholine (ACh)

- ACh is a neurotransmitter: An endogenous chemical that transmits signals across a synapse from one neuron to another.

- ACh (and glutamate) are the two most common excitatory neurotransmitters in our bodies.

- ACh signaling is critical to:
  - Muscle contraction
  - Memory & learning
  - Touch, vision, hearing, etc. (somatosensory system)

- ACh binds to ACh-receptors, which conduct sodium across the membrane.
  - If depolarization passes threshold, the muscle fiber contracts.



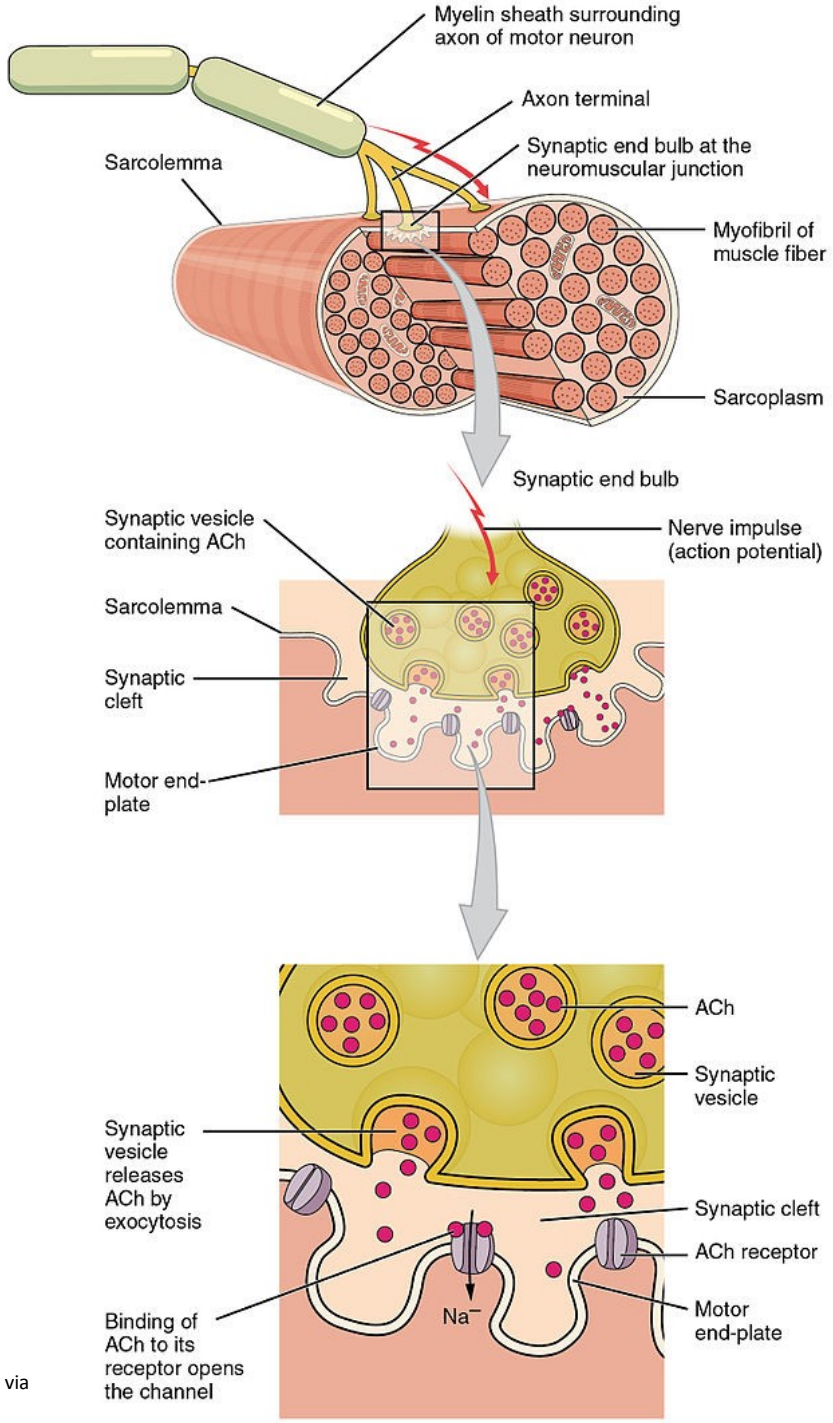
End-Plate Potentials (EPPs)

End-plate = Postsynaptic membrane of a neuromuscular junction (NMJ).

EPP = Depolarization of skeletal muscle fibers caused neurotransmitters binding to the postsynaptic membrane of the NMJ.

Miniature EPPs (Min.EPPs) = Discrete, sub-threshold depolarization events (<1mV).

Shot effect = Membrane potential "noise."



What Did They Know?

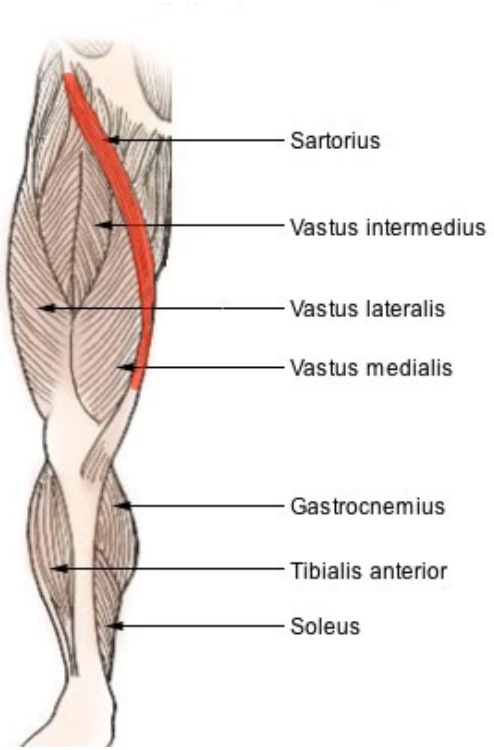
- ACh is a neurotransmitter that activates postsynaptic sodium channels.
- Katz's Nobel Prize is for his work on determining the **quantal** nature of ACh release (i.e., there is a lowest common denominator of ACh molecules released at any given time).
  - ACh is always released in multiples of 5000 (quantal). *(They didn't know this.)*

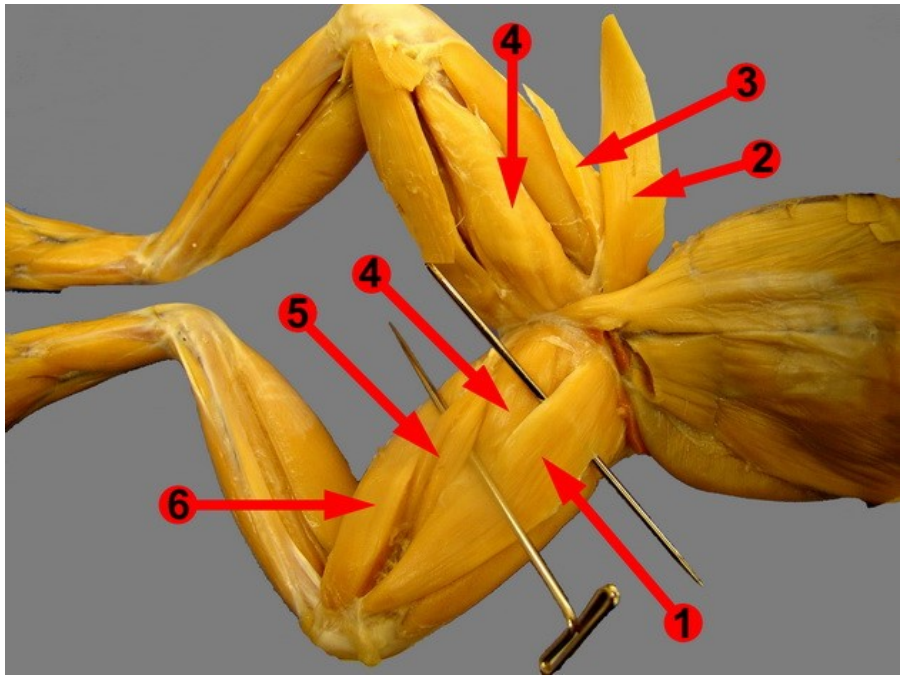
- There is membrane potential "noise" associated with ACh activity.

What Did They *Want* to Know?

- The amplitude and time course of ACh-induced noise.
- How a competitive inhibitor (curare) and anti-esterase (prostigmine) affect noise amplitude and duration.
- If a “cholinomimetic” (carbachol) has similar noise characteristics to those observed with ACh treatment.

Model and Methods





- Membrane potential recorded via:
  - Low gain amplifier measuring average level of depolarization.
  - High gain amplifier measuring voltage fluctuation.

Figure 1: ACh “noise”

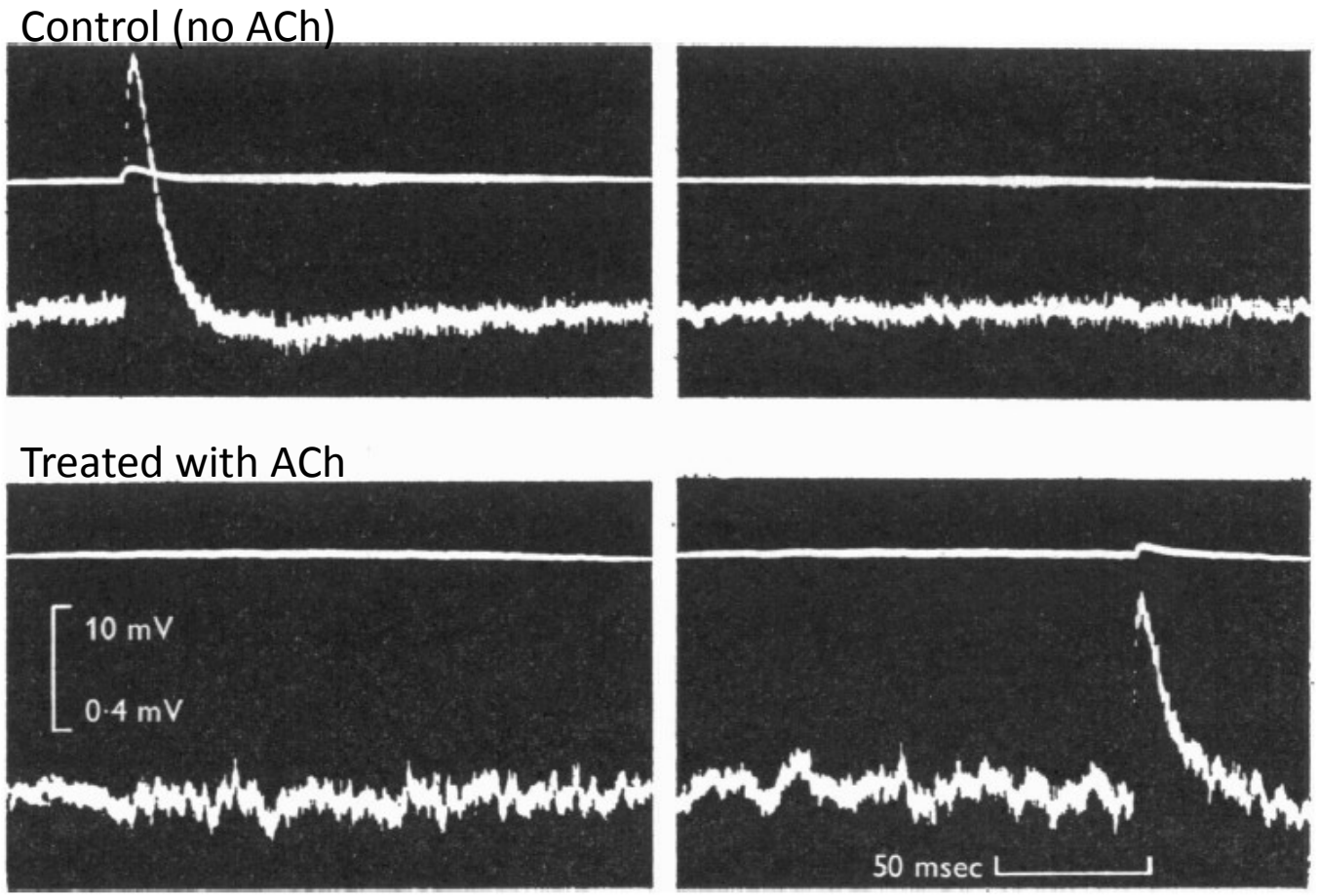


Fig. 1. Intracellular recording from an end-plate in frog sartorius. 21° C. In each block, the upper trace was recorded on a low-gain d.c. channel (scale 10 mV); the lower was simultaneously recorded on a high-gain condenser coupled channel (scale 0·4 mV). The top row shows controls (no ACh); the bottom row shows membrane noise during ACh application, by diffusion from a micropipette. In the bottom row of records, the increased distance between the low and high gain traces is due to upward displacement of the d.c. trace because of ACh-induced depolarization. Two spontaneous min.e.p.p.s are also seen.

Treatment of end-plates with ACh solution results in “noise” signature that is usually ignored.

ACh-induced “noise” on membrane potential occurs regardless of:

- Distance of pipette from end-plate
- Application of ACh solution (topical or bath)

This noise “arises from the action of ACh on end-plate receptors and not from an artifact…”

- **Fluctuations in “reaction rate” and “current pulses” (shot effects).**

Figure 2: Distribution of noise variances

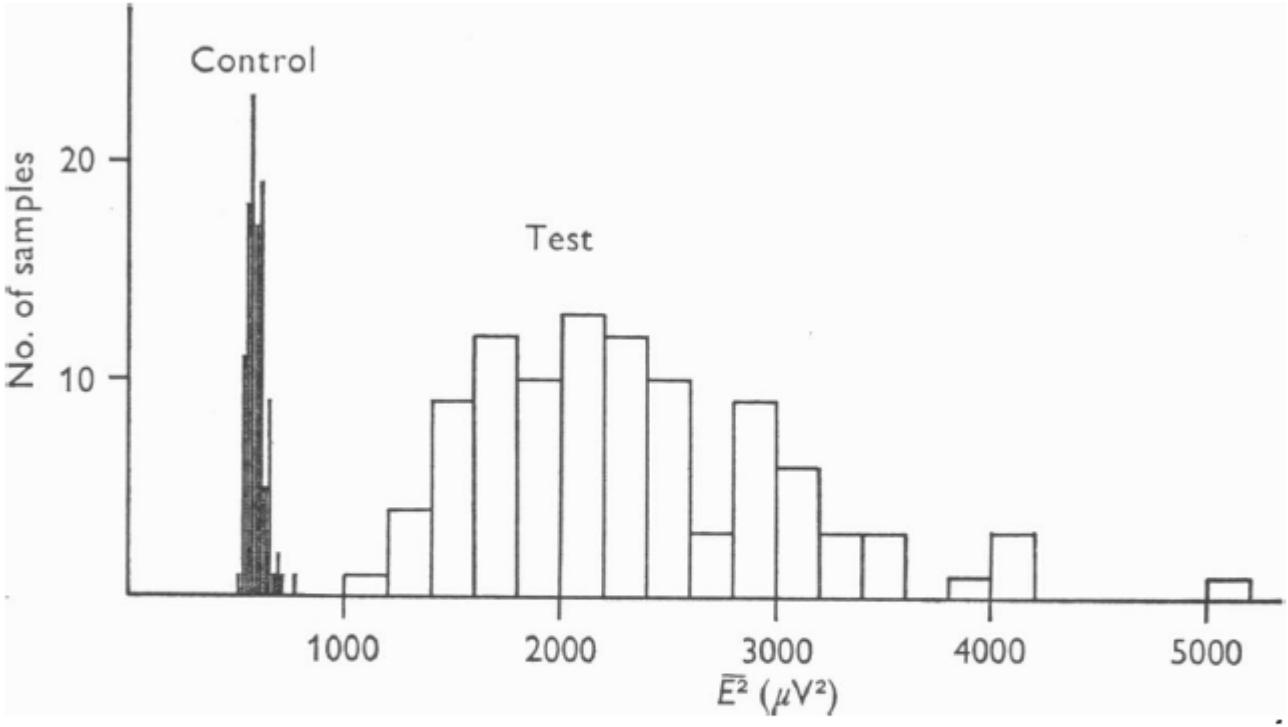


Fig. 2. Distribution of noise variances ($\overline{E^2}$ in μV^2) in control and ACh samples from a single run. The average depolarization during 'test' was approx. 10 mV. Abscissa: noise variance, in μV^2. Ordinate: number of samples (of 0·25 s length). Temp. 2° C. Control samples were collected, as usual, before as well as after the ACh test.

There is a lot more noise variance after ACh treatment than without.

There is wider distribution of noise variance in ACh treatment than without.

Figures 5 & 6: Noise-Potential Relationship

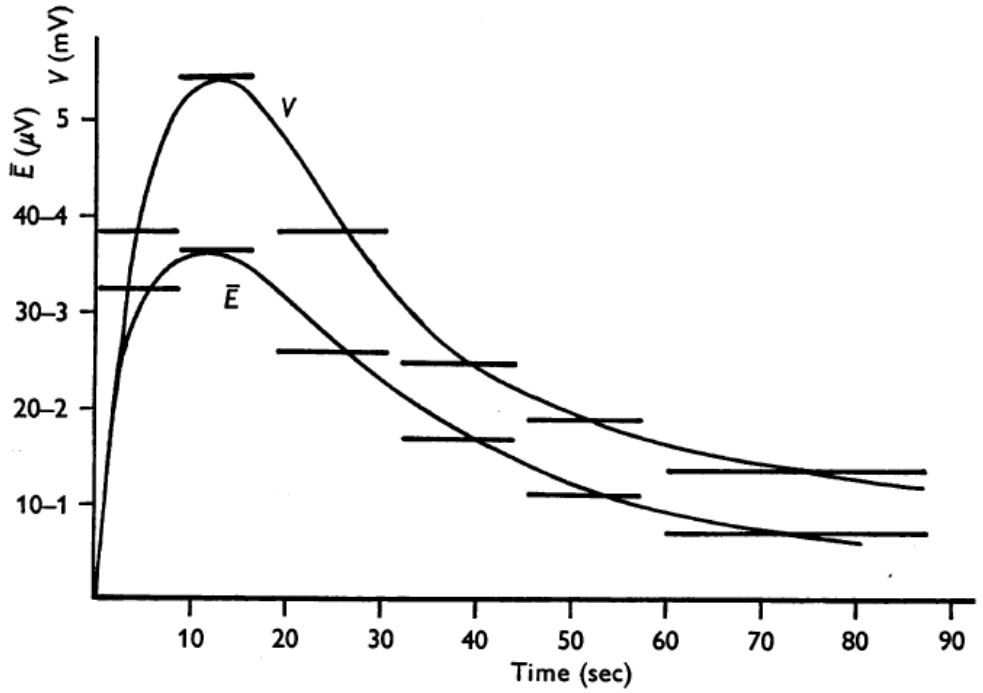


Fig. 5. ACh-induced depolarization (V in mV) and noise ($\bar{E}_{r.m.s.}$ in μV) during gradual increase and withdrawal of the drug. Temp. 11° C. Abscissae: time from start of ACh application. See also Table 1. Horizontal bars show the average levels of V and $\bar{E}_{r.m.s.}$ during individual sampling periods.

Membrane potential (V) and noise (E) are related, but their relationship changes over time after ACh addition.

Figures 5 & 6: Noise-Potential Relationship

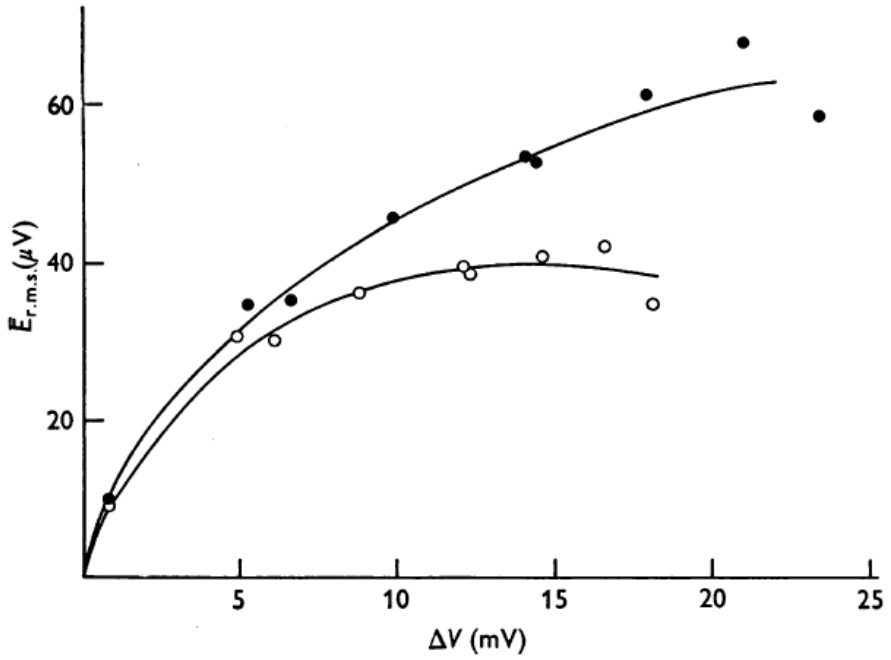


Fig. 6. Relation between ACh potential (V in mV) and ACh noise ($\bar{E}_{r.m.s.}$ in μV). Temp. 4° C. Open circles: uncorrected values. Filled circles: values corrected for non-linear summation (using eqns. (8) and (9).

Membrane potential (V) and noise (E) are related, but their relationship changes over time after ACh addition.

Noise level is also related to changes in membrane potential.

Figure 8: Amplitude Relationship

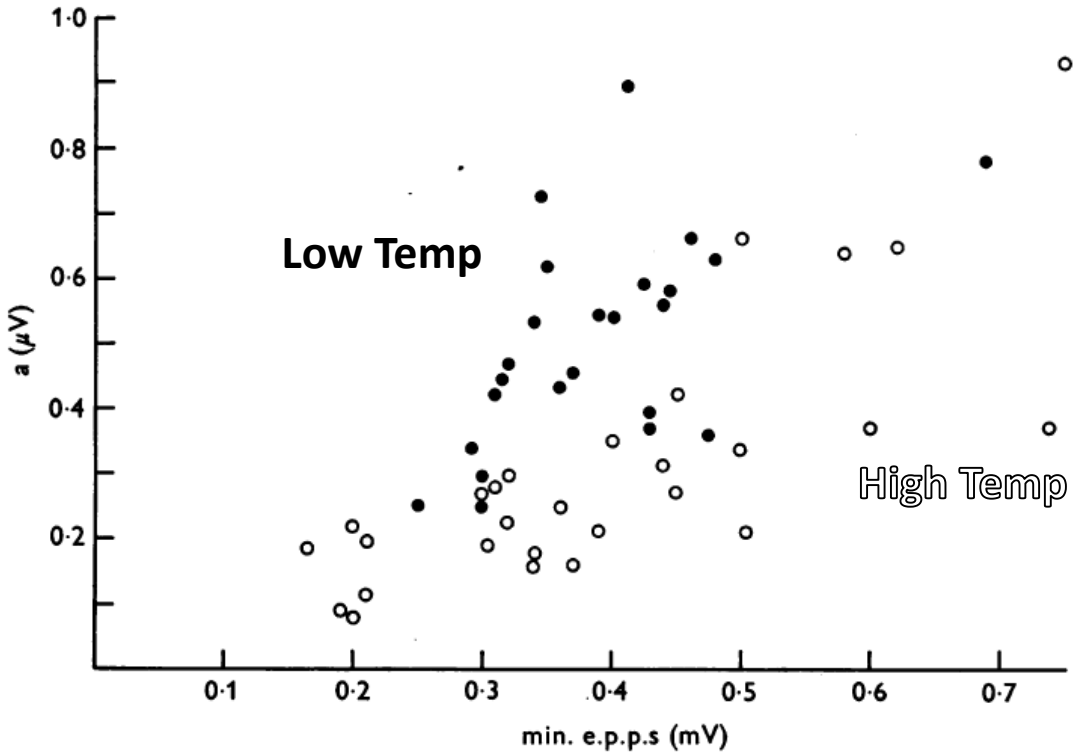


Fig. 8. Relation between mean amplitudes of min.e.p.p.s (abscissae) and values of a (ordinates). Results have been separated into two groups, high temperature (open circles) and low temperature experiments (filled circles). See also Table 2. In both groups there is a highly significant correlation between the two variates (correlation coefficients are +0·595 in twenty-four low temperature experiments, and +0·801 in twenty-nine high temperature experiments).

The amplitude of min.EPPs is related to noise amplitude (a).

The noise amplitude increases as temperature decreases, but the min.EPP amplitude doesn't change…

Thoughts?

Effects of Competitors, Mimics, etc.

- **Effect of curare**: min.EPP amplitude is *highly* reduced (>90%), but noise amplitude does not change.

  Does this make sense?

- **Effect of prostigmine**: min.EPP amplitude increases by 55%, but noise amplitude only increases slightly.

  Does this make sense?

- **Effect of carbachol**: next slide

Figure 15: Carbachol Noise vs. ACh Noise

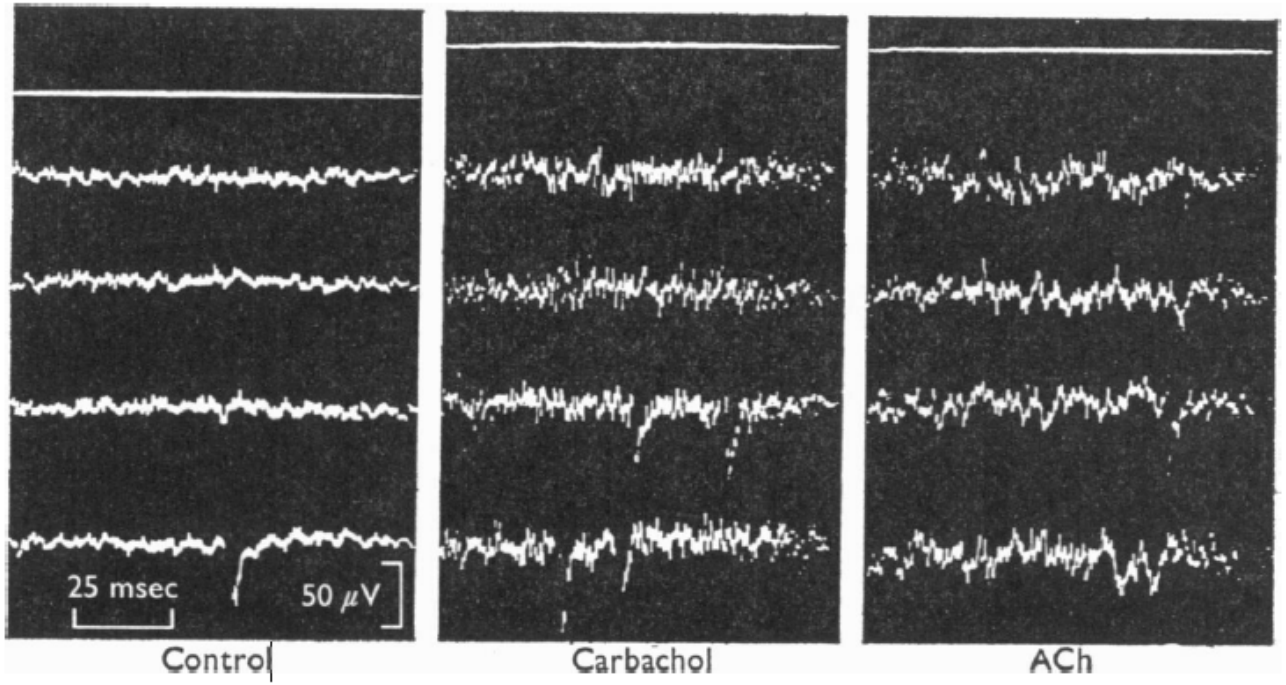


Fig. 15. Sample records (magnetic tape playback) of focal extracellular noise during Carb and ACh applications. Temp. 21° C. Membrane depolarization (about 5 mV) was monitored by intracellular recording and is indicated by the upward displacement of the horizontal line at the top of each column. The very large rapid deflexions (e.g. in the bottom traces of control and Carb records) arose from external min.e.p.p.s which saturated the tape amplifier.

Noise after carbachol treatment is "faster" than ACh noise.

This is attributed to shorter duration of ion channel open-state (0.3ms compared to 1ms with ACh).

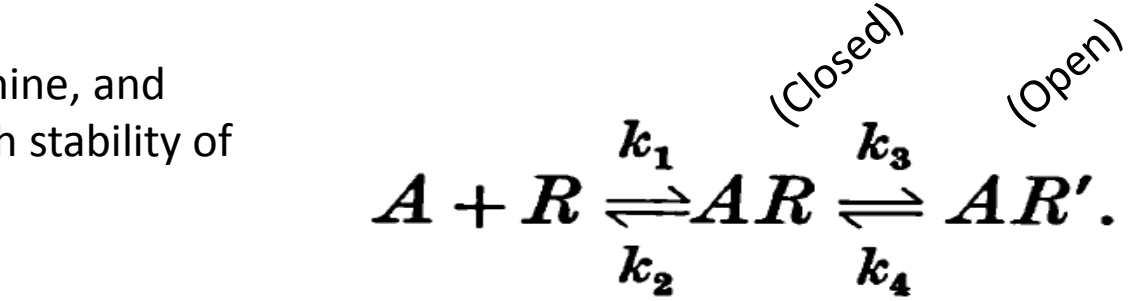
Carbachol noise also has smaller amplitude (which makes sense with previous point).

Discussion

- Authors openly acknowledge that these experiments were “exploratory” and their analyses would need revisions.
- Measuring noise amplitude and timecourse opens up new ways to investigate:
  - The number of ionic gates involved in min.EPPs
  - The absolute conductance of single ion channels
  - The duration of the “gating” action
  - The total transfer of charge through ion channels
  - The relationship between noise timecourse and binding kinetics
  - The probability of ACh molecules acting multiple times

Discussion

Their results with curare, prostigmine, and carbachol could all have to do with stability of receptor-ligand interactions.



Significance

- Specific:
  - ACh-induced noise lasts ~1ms and its net transfer charge corresponds to about 50,000 sodium ions.
  - ACh-induced noise increases in duration and amplitude at lower temperatures.

- General:
  - Noise in your data can be meaningful.
  - Math is a powerful biological tool, when applicable.
  - Electrophysiological data contains much more information than I thought.