Neurotransmitters, quantal synaptic release & synaptic vesicles

papers by B. Katz and Heuser
Outline

- Nobel Lecture paper (Katz) and biological context
- Statistical observation of neurotransmitter ‘shot noise’
- Heuser’s experiment + pictures
Neurotransmitter Acetylcholine (ACh)

- Neurotransmitters are molecules that transmit signals from a neuron's axon terminal to a target cell (across the synaptic cleft)
- ACh is a neurotransmitter used in both peripheral (PNS) and central (CNS) nervous system
  - Induces muscle contraction in PNS
  - First identified neurotransmitter
  - Ester of acetic acid and choline
Neuromuscular Junction

- Synapse between motor neurons and muscle cells in PNS

Axon terminal of motor neuron (pre-synaptic)

- End-plate region of muscle fiber serves as ‘chemo-electric transducer’ for ACh
- Binding of ACh opens up channels through which ions can pass — leading to depolarization of membrane
Action Potential

- Resting potential of end plate ~ -100mV with firing threshold of ~ -65 mV

- Action potential occurs when enough ACh released from axon terminal to induce end-plate depolarization at threshold level
  - Binding of ACh opens ligand-gated ion channels (Na+ and K+)
  - When depolarization threshold met, voltage-gated ion channels in postsynaptic membrane open for further depolarization ("all-or-nothing")
Bernard Katz

- Nobel Prize in 1970 for “discoveries concerning the humoral transmitters in the nerve terminals and the mechanism for their storage, release and inactivation”
  - Shared with J. Axelrod (US) and U. von Euler (Sweden)

- Method of ‘intracellular recording’

- Studied neuron->acetylcholine->muscle system

\[ N \rightarrow ACh \rightarrow M \]
On the Quantal Mechanism of Neural Transmitter Release

Experiments performed using frog sartorius

Proximity to sciatic nerve
  - Large nerve that runs from lower back to lower limb
  - Thousands of axon terminals – motor neurons were of specific interest
Quantal release of ACh

- **Top:** intracellular recording in muscle fiber (no stimulus)
  - Shows localized nature of miniature end-plate potential spikes (min. e.p.p.) – ‘the quanta’

- **Bottom:** same muscle fiber – nerve impulse

Fig. 1. Spontaneous "miniature end-plate potentials". (From ref. 1) A: intracellular recording at an end-plate. B: recorded 2 mm away in same muscle fibre. Upper portions were recorded at low speed and high amplification (calibrations 3.6 mV and 46 msec): they show the localized spontaneous activity at the junctional region. Lower records
Quantal release of ACh

- Action potential formed only if sum of min e.p.p. (the quanta of ACh molecules) exceeds firing threshold of membrane
  - Amplitude of min e.p.p.~0.5 mV (threshold ΔV ~ 35 mV)
Quantal release of ACh

- Action potential formed only if sum of min e.p.p. (the quanta of ACh molecules) exceeds firing threshold of membrane
  - Amplitude of min e.p.p. \( \sim 0.5 \text{ mV} \) (threshold \( \Delta V \sim 35 \text{ mV} \))

- Katz discovered that depolarization of presynaptic axon terminal causes rate of quanta discharge to dramatically increase

---

Fig. 2. Electrical control of the frequency of min.e.p.p.’s (cf. ref. 14). In each pair of traces, the upper shows miniature potentials, the lower indicates current flowing through the terminal part of the motor axon. The cathode was placed near the junction so as to depolarize the nerve endings. This caused the frequency of the discharge to increase...
Role of Calcium

- Depolarization of membrane potential not alone sufficient to increase ACh discharge frequency
- Presence of ionic calcium in external medium necessary for ACh discharge

For constant membrane polarization, Katz observed drastic difference in activity at different Ca$^{2+}$ levels.

- # of pulses observed:
  - A (0 of 6)
  - B (3 of 6)
  - C (5 of 6) – some multiple
Summary of Nobel paper

- Neurotransmitter ACh released in quanta (later known to be stored in synaptic vesicles) even without neural stimulus
  - Electrical observation of pulses on end-plate – uniform size/shape
  - Presence of min.e.p.p. found in diverse synaptic systems
- Frequency of release is directly related to depolarization of presynaptic membrane and Ca\textsuperscript{2+} concentration
  - Need higher frequency of release to induce action potential at muscle membrane
Summary of Nobel paper

- Neurotransmitter ACh released in quanta (later known to be stored in synaptic vesicles) even without neural stimulus
  - Electrical observation of pulses on end-plate – uniform size/shape
  - Presence of min.e.p.p. found in diverse synaptic systems
- Frequency of release is directly related to depolarization of presynaptic membrane and Ca\(^{2+}\) concentration
  - Need higher frequency of release to induce action potential at muscle membrane
- Questions remain:
  - What is the effect of one ACh molecule at the end-plate?
  - Can we see these quanta (vesicles) by direct observation?
1972 paper by Katz and Miledi:

*The Statistical Nature of the Acetylcholine Potential and its Molecular Components*

Characterization and analysis of ACh noise

- Shot noise (statistical fluctuations due to finite number of particles)

Experiment:

Intracellular recording of muscle membrane potential changes from application of ACh in 30-60s durations

Readout to 565 Tek scope w/ 2 levels of amplification sent to tape. Digitized with LINC-8 cpu.
ACh noise

- Increase in membrane voltage noise observed after ACh application
- Extra noise attributed to ACh (eliminated other factors)

![Graph showing control and ACh applied](image)

**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
ACh noise quantified

- Histogram of noise variance → from previous
  - Net noise variance = 43 µV for ACh test sample depolarized @ 10 mV
  - 100 samples taken at 1 ms intervals with 0.25s windows

- If noise is in fact ‘shot noise,’ should follow Poisson statistics
  - Variance directly proportional to mean
  - Assumes linear dose/response relationship

Fig. 2. Distribution of noise variances ($\overline{E^2}$ in µV²) in control and ACh samples from a single run. The average depolarization during ‘test’ was approx. 10 mV. Abscissa: noise variance, in µV². 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.
Simple theoretical model:

Suppose ACh potential $V$ is made up of linearly additive ‘shot effects’ $f(t)$, randomly occurring with average frequency $n$ such that:

$$V = n \int f(t) \, dt$$

with noise variance given by:

$$\overline{E^2} = n \int f^2(t) \, dt.$$ 

Assume $f(t)$ takes the form: $f(t) = a e^{-t/\tau}$.

- Pulse with amplitude $a$ and zero rise time
- Decay time constant $\tau$

Giving the following relationship between variance and mean

- Linear relationship with slope $= a/2$
Using model + previously shown data, amplitude $a$ of elementary shot effect $\sim 0.4$ µV.
- 3 orders of magnitude less than min. e.p.p
- Shot effects only observable during high frequency fluctuations.

Experimenters also wanted to show linear relationship between mean and variance
- Do this by measuring noise variance as a function of ACh depolarization
- Noise RMS ($\sqrt{\text{variance}}$) as a function of ACh depolarization:

![Graph showing noise RMS as a function of ACh depolarization]

- Note: open circles show uncorrected data. Black dots show data corrected for non-linear summation.

Fig. 6. Relation between ACh potential ($V$ in mV) and ACh noise ($E_{\text{rms}}$ in $\mu$V). Temp. 4°C. Open circles: uncorrected values. Filled circles: values corrected for non-linear summation (using eqns. (8) and (9)).
ACh noise quantified

- Noise RMS as a function of $\sqrt{\text{ACh depolarization}}$:
  \[ E^2 = \frac{V a}{2}. \]

  ![Graph showing noise RMS as a function of ACh depolarization](image1)

  ![Graph showing replotting of corrected values](image2)

- Linear relation as expected for shot noise – slope gives shot amplitude $= 0.42 \mu V$
ACh noise – nonlinear effects

- Addition of ACh induces conductance (ion channels opening) in membrane
  - Individual conductances do not contribute linearly to overall membrane potential – saturation effects

![Circuit diagram]

\[
\frac{V}{V_0} = \frac{gR}{1+gR},
\]

Fig. 3. Circuit diagram representing input resistance \((R)\) and resting potential \((E_r)\) at end-plate region of muscle fibre shunted by variable ACh-induced conductance path \((g)\), with ‘equilibrium potential’ \(\epsilon\).

- Gives correction to mean-variance relation:
  \[
  \bar{E}^2 = \frac{V_0 g R a}{2(1+gR)^4} = \frac{V a}{2(1+gR)^3}.
  \]
ACh noise paper summary

- Paper further describes temperature and muscle fiber size dependence of shot noise amplitude
  - Amplitudes range from 0.1 - 1 µV
- Also discusses temporal characteristics of shot noise (variance as function of frequency)
- Showed evidence of ACh shot effects underlie both noise and average depolarization
- From observations, authors derive some physical properties of the ‘ion gate’ in the membrane
Noise effects $10^3$ less than min.e.p.p
- Gives conductance change of $10^{-10} \, \Omega^{-1}$
- Using amplitude values of shot effects over time of 1 ms estimated each ACh molecule transfers $10^{-14} \, \text{C}$ or roughly $5 \times 10^4 \, \text{ions}$

Also estimated $\sim 1000$ ion gates opened per ACh quanta by noting ratio of average min.e.p.p. to shot amplitude

Number of ACh molecules/quanta still unknown – estimates of 50,000
- Suggests high inefficiency
1979 Paper by Heuser et al.
- Synaptic Vesicle Exocytosis Captured by Quick Freezing and Correlated with Quantal Transmitter Release

- **Synaptic Vesicle** – storage/transport ‘containers’ for neurotransmitter quanta
  - First direct observation of vesicle-quanta association

- **Exocytosis** - process by which vesicles are discharged out of presynaptic membrane, releasing neurotransmitter quanta

- Experimental apparatus freezes tissue a few ms after stimulation of neuromuscular junction
Heuser apparatus

- Tissue mounted on freezing head, suspended above a block of Cu cooled to 4 K.
Heuser apparatus

- Tissue mounted on freezing head, suspended above a block of Cu cooled to 4 K.
- Once released, tissue passes through ‘stimulating wires’
  - Triggered by magnetically activated relay switch
Heuser apparatus

- Critical requirement of apparatus is accuracy of stimulation-freezing timing
  - ± 0.2 ms accuracy attained
  - Time intervals of 0-0.5 seconds possible
- Once frozen, tissue transferred to liquid nitrogen, pumped down in vacuum chamber and ‘fractured’
  - Create carbon-metallic replica of tissue
  - Observe with TEM
Structural Changes after Transmitter Release at the Frog Neuromuscular Junction

- Nerve terminals at rest
  - Synaptic vesicles visible

Figures 1 and 2: High magnification views of freeze-substituted nerve terminals at rest (Fig. 1) and stimulated in 4-AP at 5.2 ms before freezing (Fig. 2). Many pockets occur in the stimulated nerve terminal, which is sectioned through an active zone. The smaller of these pockets have the same diameter and curvature as synaptic vesicles. The larger, shallower pockets look like collapsing vesicles. × 180,000.
Images taken with increasing stimulation-freezing times

3.7 ms

3.8 ms

5.2 ms

20 ms

50 ms

250 ms
Close up of synaptic vesicle opening

2 types of synaptic vesicle opening fracturing (20ms after stimulation)
Close up of synaptic vesicle opening sequence

**Figure 27** Montage of six different forms of vesicle exocytosis, arranged to portray the sequence of vesicle fusion and coalescence with the plasma membrane suggested by the data in this paper. Actual stimulation-freezing intervals were as follows: (a) 3.7 ms; (b) 5.2 ms; (c) 5.2 ms; (d) 5.2 ms; (e) 20 ms; (f) 50 ms. X 300,000.
Summary

- Acetylcholine is neurotransmitter used in motor control

- Neurotransmitters released in quanta known as synaptic vesicles
  - Observed indirectly via min.e.p.p in neuromuscular junction and later directly via imaging

- ACh noise analysis shows underlying particle nature of neurotransmission
Rate of freezing determined by observing attenuation of AC signal

- Tissue @ room temp is primarily resistive
- Freezing/Frozen tissue acts as varying capacitor between two metal plates – AC impedance indicative of freezing progress