Responses of Retinal Rods to Single Photons

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Introduction

- Companion Paper to Baylor et.al. (1979)

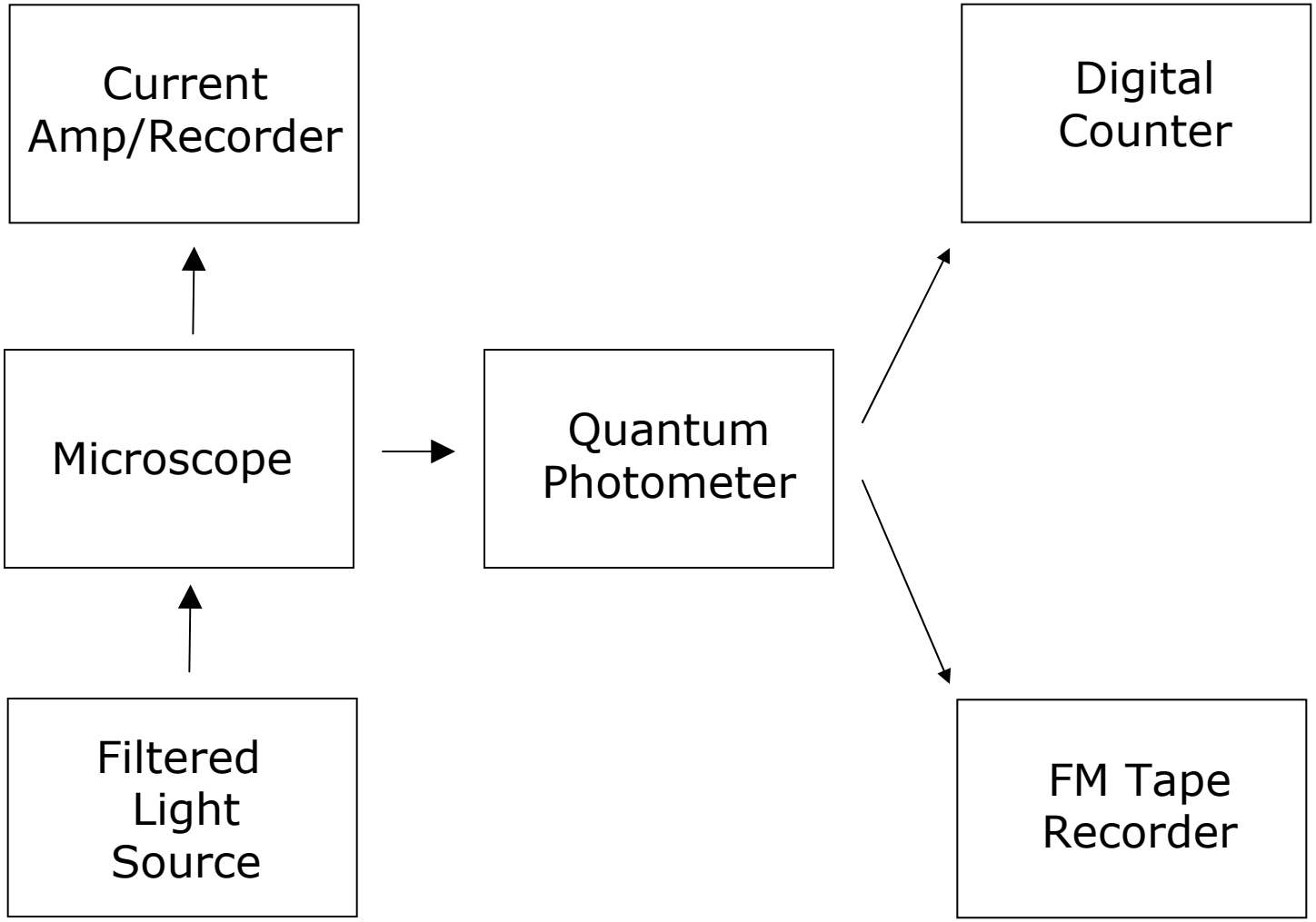
  Empirically described the general properties of membrane current of rod outer segments

  Baylor et.al, *The membrane current of a single rod outer segments.* J. Physiology, **288**:589-611, 1979

- Hypothesis:

  The fluctuations in photocurrent during presentation of dim light arise from the quantal nature of light.

Methods


Current Amp/Recorder
Digital Counter
Microscope
Quantum Photometer
Filtered Light Source
FM Tape Recorder

Methods

- Suction Electrode Measured Membrane Current of a Single Rod Outer Segment
- Light at 520 nm Was Used
  - maximize rhodopsin isomerization
  - minimize metarhodopsin III isomerization (side product)
- Light at 580 nm Was Used as Control
- Signal Processing
  - Power Spectra
  - Amplitude of Response to Dim Flashes
    - Difference Method
    - Least Squares Method

Suction Electrode

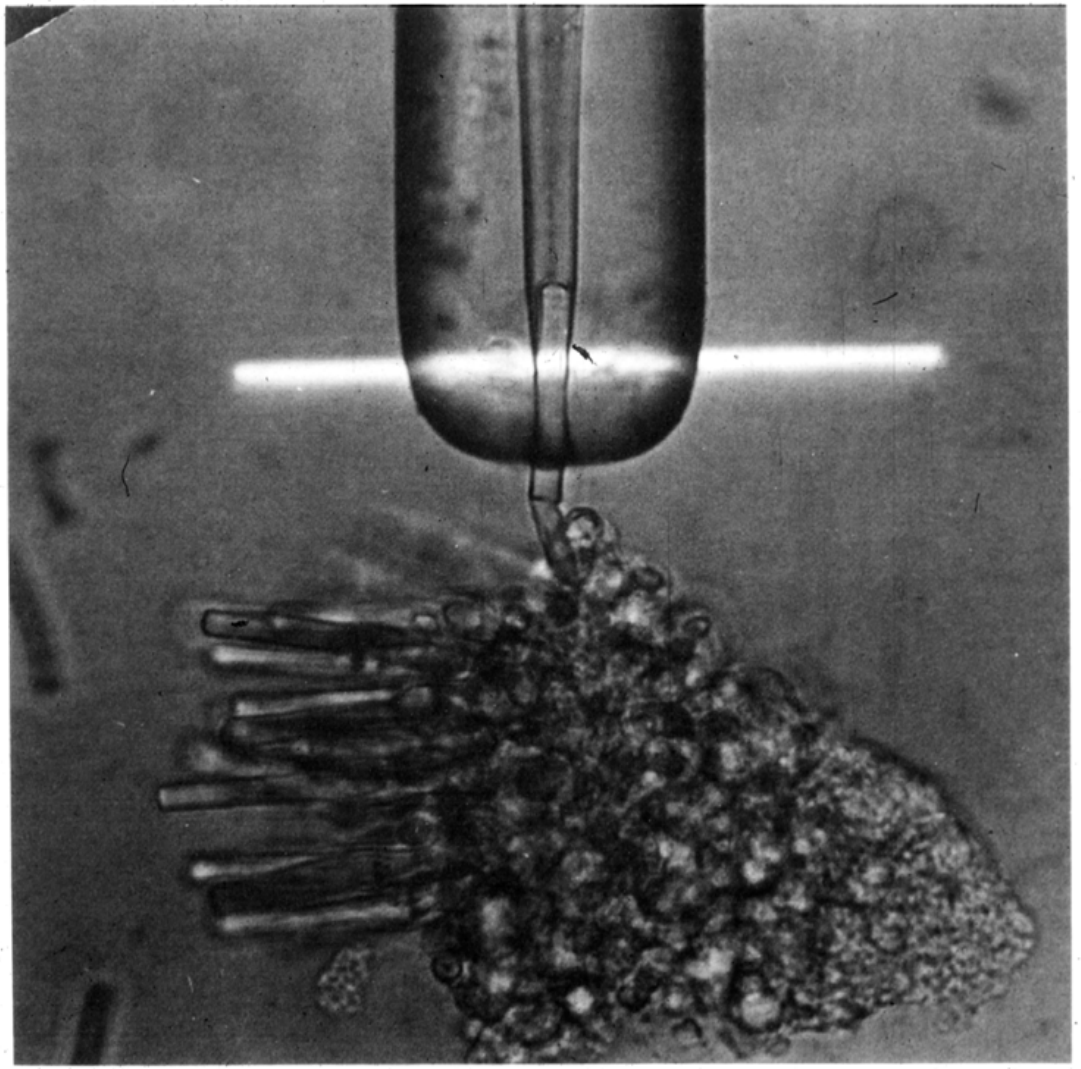
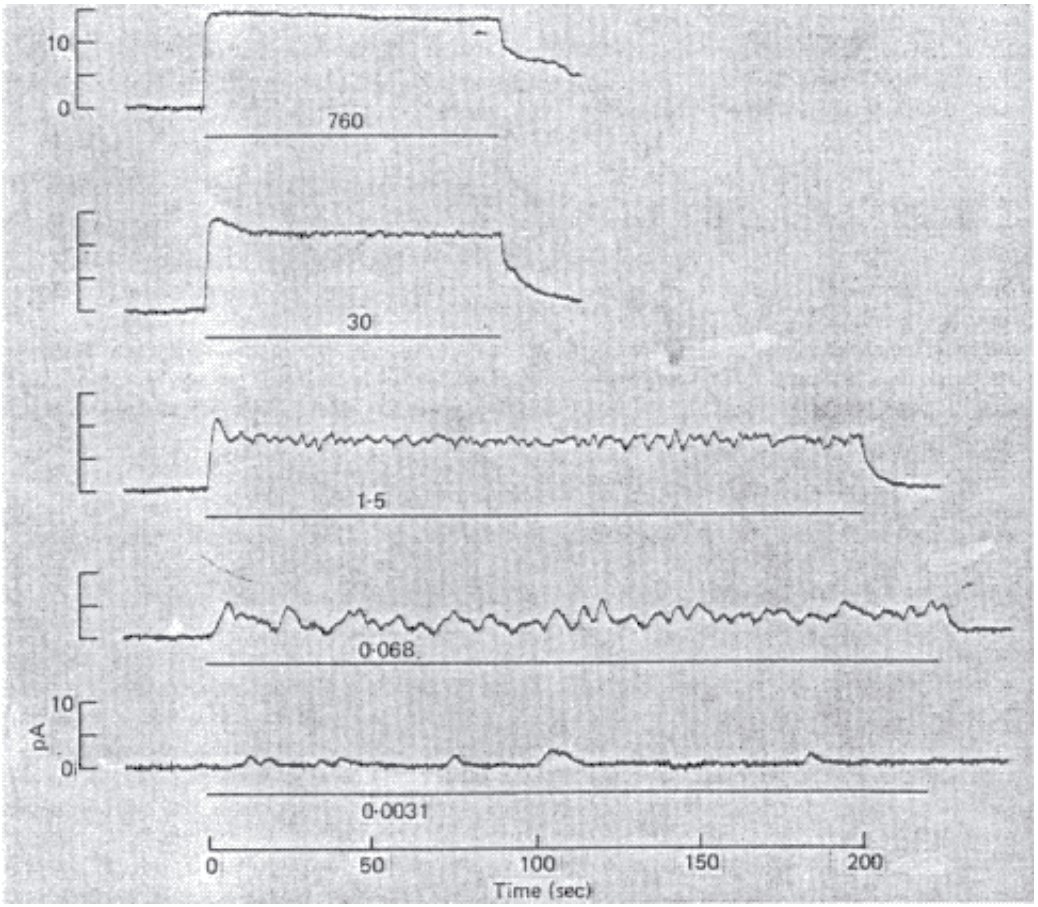


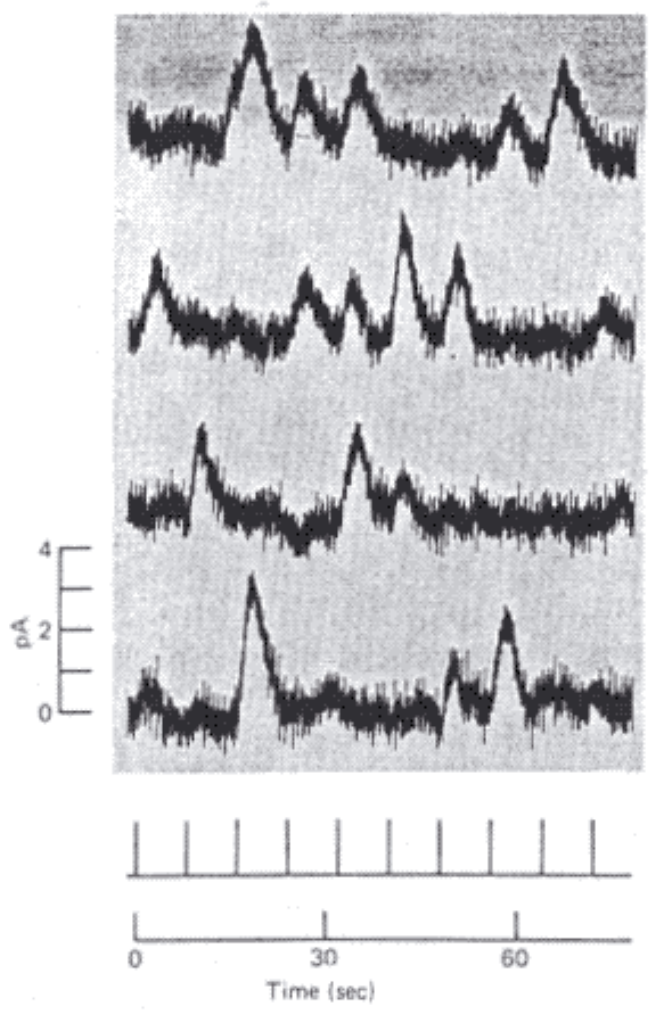
Figure 1



- Characterization of Photocurrent to Various Steady Light Intensities and Durations
  - Number = Intensities (photons/(μm^2 s))
  - Bar = Period of Stimulus (seconds)
- Fluctuations Appear at Lower Intensities
- Fluctuations Disappear at Higher Intensities
- Similar to Previous Work which Suggests Fluctuations Are Due to Quantal Nature of Light.

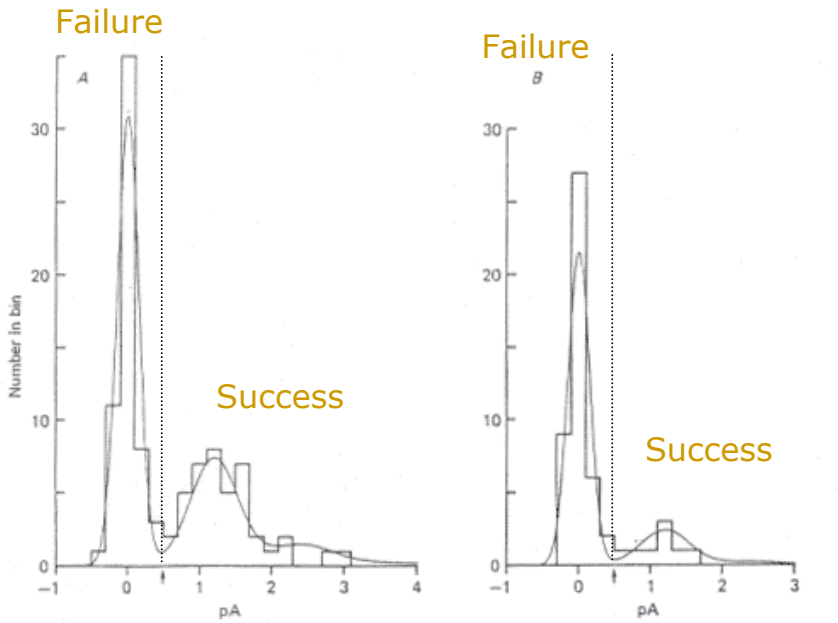
  (Fuortes & Yeandle, 1964)

Figure 2



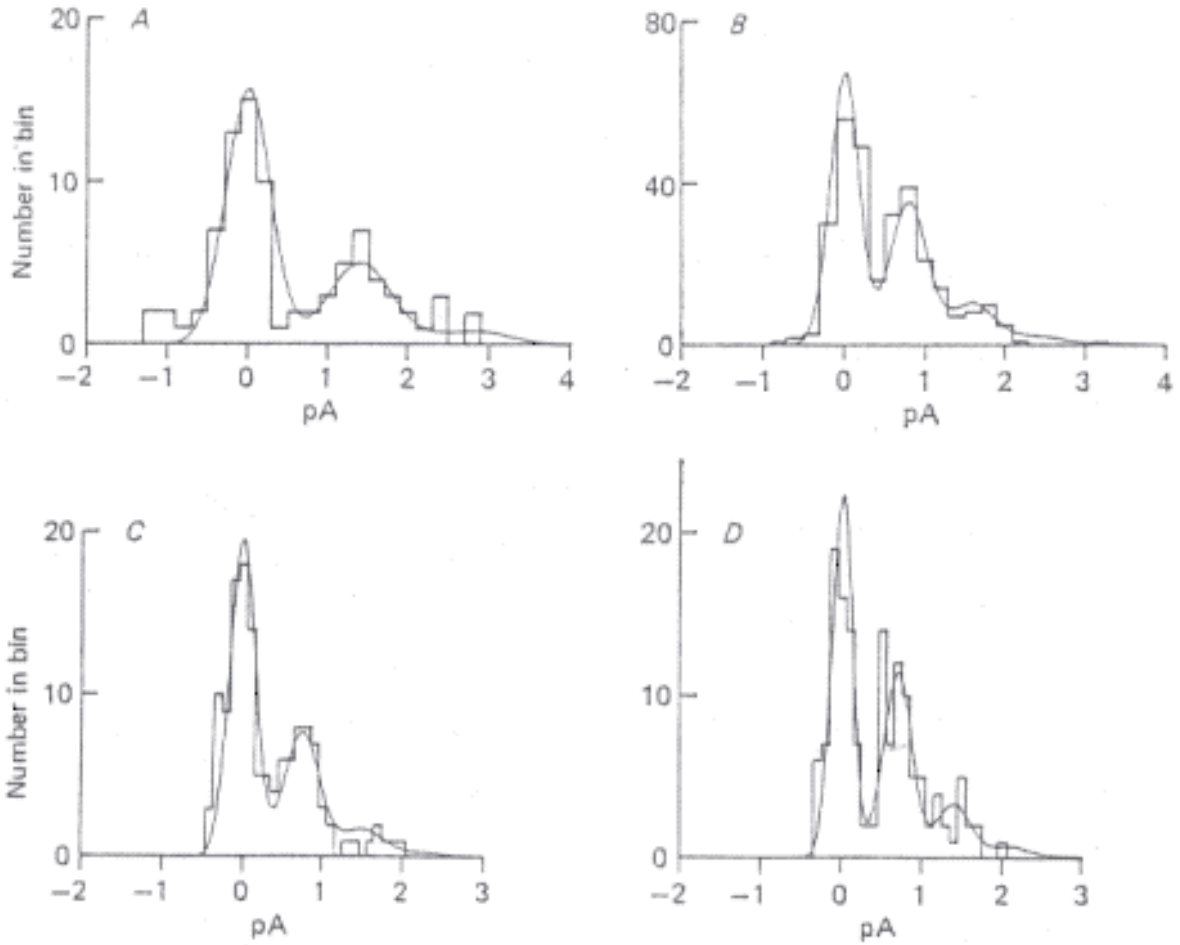
- Fluctuations in the Response to Impulses of Light
- Three Distinct Amplitudes Were Observed from the Presentation of Impulse Stimuli
  - No response
  - 1 pA
  - 2+ pA
- Further Suggests a Quantal Nature of Photocurrent Production

Figure 3



- Histogram of 99 responses
- Bimodal Population Seen
- Figure 3a
  - 58/99 resulted in failure (0.5 pA cutoff)
- Figure 3b
  - Same cell at lower intensity
  - 44/99 resulted in failure (0.5 pA cutoff)
- Assuming Poisson-Distribution ($p_k = e^{-m}m^k/k!$), the Mean Number of Events per Trial = 0.53

Figure 5



- Amplitude Histograms of Dim Flash Responses from Four Cells
- One Third of Cells Failed to Show Obvious Quantization
- Due to Low Amplitude / High Variance System
- Line Fitting: Gaussian-Poisson Hybrid.

Figure 6

- Is This Caused by a Single or Multiple Isomerizations?
- Test if the Mean Number of Photoisomerizations is Proportional to Light Intensity.
  - Open Circles: Empirical Results;
  - Line: Theoretical assuming Single Isomerization Required.
  - Multiple Isomerization Curve Would be **TOO** Steep

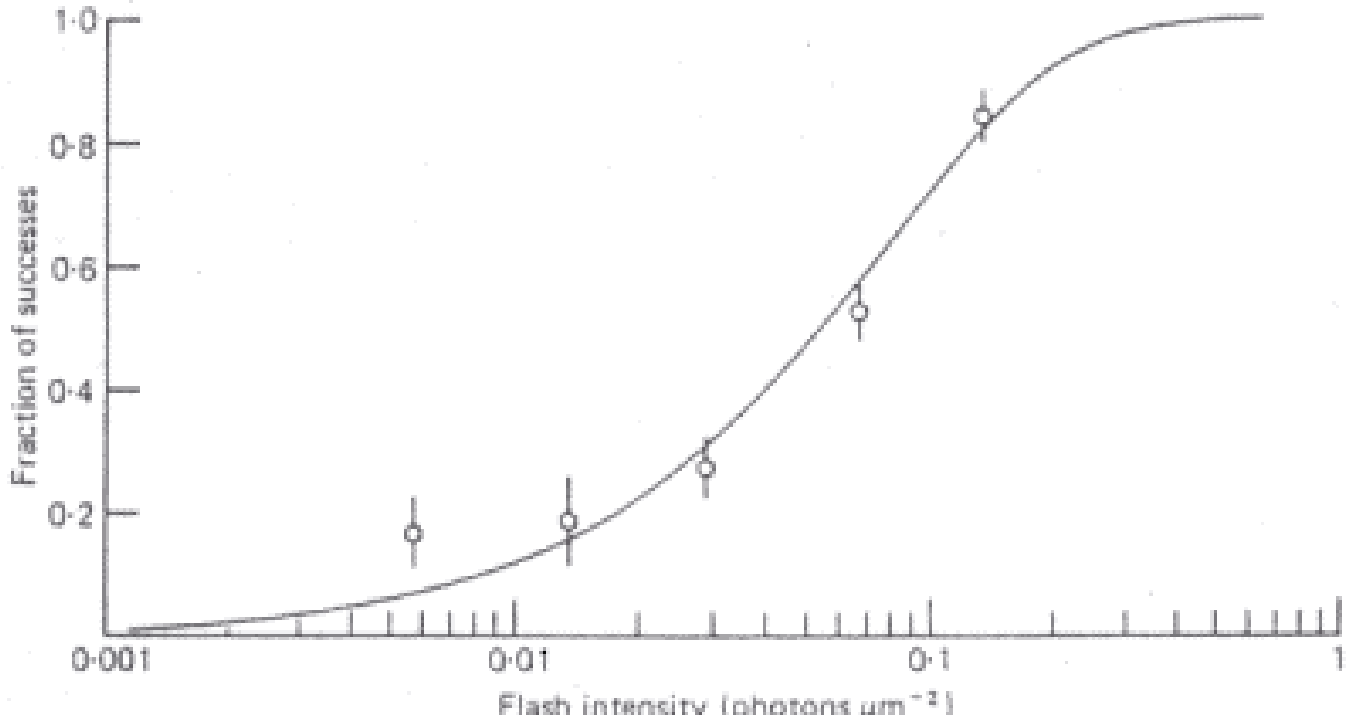
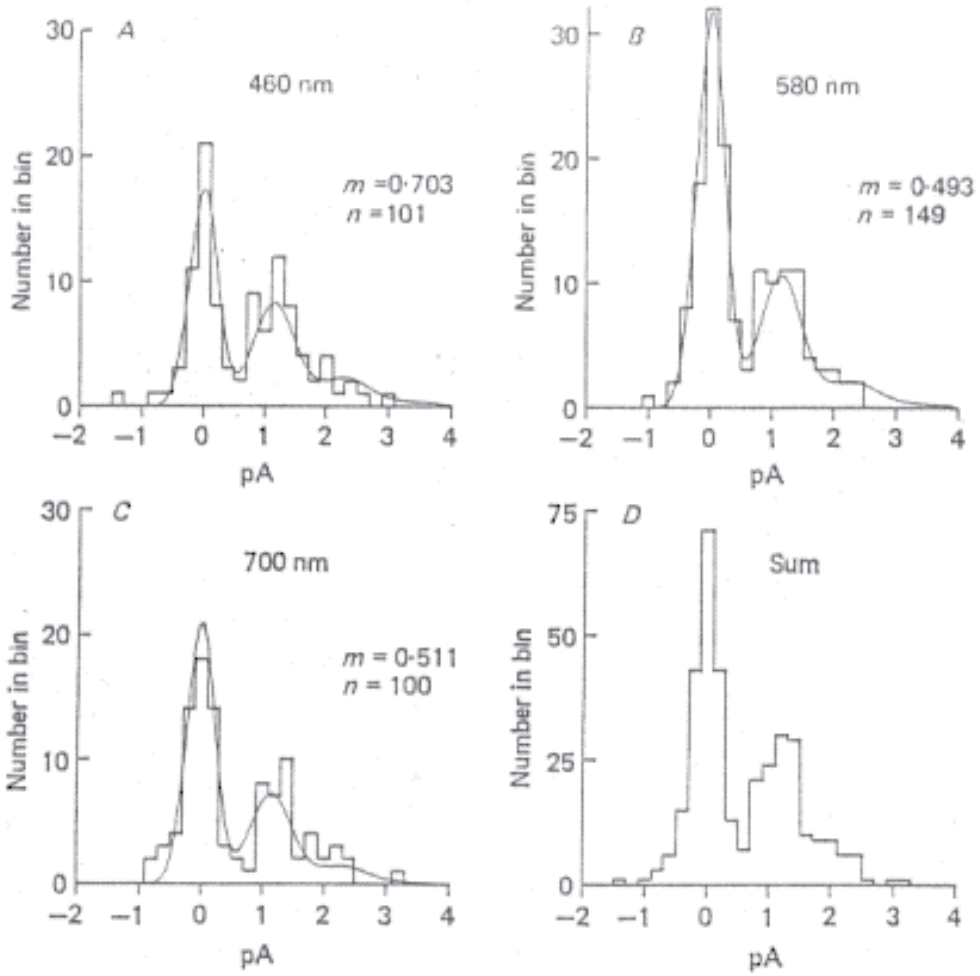
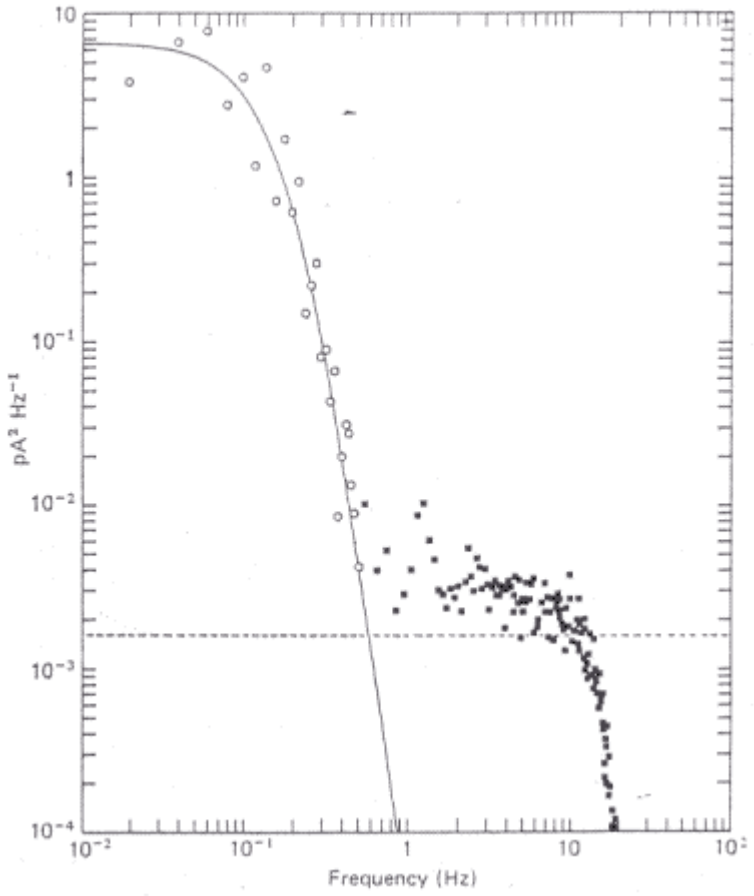


Figure 8



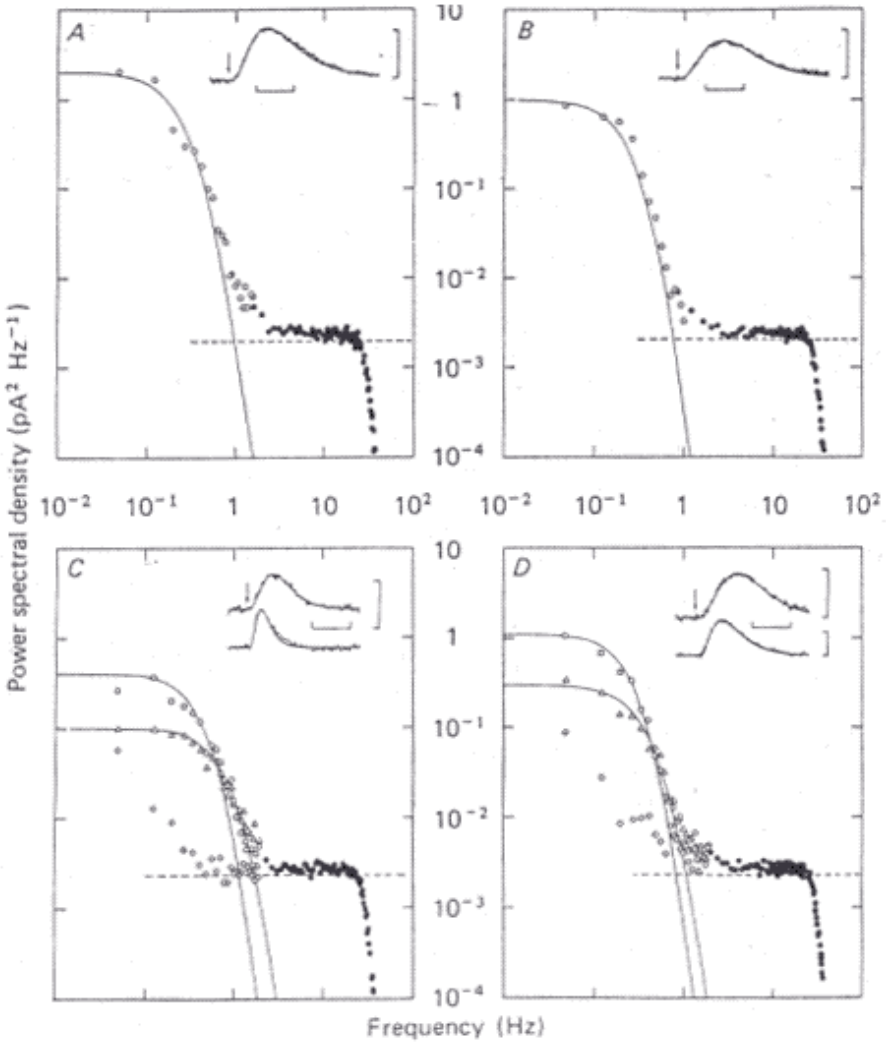
- The Size and Shape of the Response to an Absorbed Photon Seems to Be Wavelength-Invariant

Figure 9



- Interaction of Quantal Events During Steady Illumination
- Power Spectrum of Figure 1 (0.068) Shown.
- The Curve Is the Expected Power for the Superposition of Quantal Events.
- Falloff Due to 15Hz 6-pole Low Pass Filter
- Dashed Line: Thermal Noise in the Leakage Resistance between the Pipette and Cell

Figure 10



- Power Spectral Densities for Four Cells
  - ○: Dim Illumination
    - 0.2 photons/(μm²·s)
  - △: Moderate Illumination
    - 5 photons/(μm²·s)
  - ◇: Bright Illumination
    - 500 photons/(μm²·s)
- Bright Illumination Does Not Follow Predicted Curve; Adaptation

Conclusions

- Amplitude Histograms of Responses to Dim Flashes Showed Discrete Distribution; Suggests Quantized Nature.
- Variance of Fraction of Successes Consistent with Hypothesis that Each Quantal Electrical Event Resulted from a Single Photoisomerization.
- The Size and Shape of the Quantal Event was Wavelength-invariant.
- The Power Spectral Density of the Responses Matches that of Independently Occurring Quantal Events, But Breaks Down at Higher Light Intensities