Kandel's Experimental Setup

Used in recording Gill-Withdrawal Reflex in Aplysia

Introduction

– The method section of the paper describes how the researchers conducted their quantal analysis of synaptic depression in Aplysia's gill-withdrawal reflex.

A Quantal Analysis of the Synaptic Depression Underlying Habituation of the Gill-Withdrawal Reflex in *Aplysia*

(synaptic plasticity/behavior)

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1.Preparing the Experimental Setup

Animal Used: **Aplysia californica** (a type of sea slug).

Size of Aplysia: 80-200 *grams*.

Isolated Nervous System: The abdominal ganglia (a cluster of neurons controlling the gill-withdrawal reflex) were removed and kept alive in an artificial seawater solution.

Temperature Conditions: The experiments were conducted at 20-23°C.

2. Recording Neural Activity

Stimulating Neurons

- They used intracellular electrodes to stimulate single sensory neurons in Aplysia.
- These sensory neurons send signals to motor neurons that control the gill muscles.

Recording Responses

- The researchers recorded the electrical signals from the motor neurons to see how strongly they responded to sensory neuron activation.

- The motor neurons tested were

- L7 (main gill motor neuron).
- LBS-3 (siphon motor neuron).
- L16 (an interneuron).

Electrode Setup:

- Single electrodes (low resistance, <5 MΩ) were used to stimulate and record from sensory neurons.
- Double electrodes (low resistance) were used to record from the follower cells (motor neurons).

Signal Processing

- The recorded signals were amplified and analyzed using:
- A cathode follower circuit (to stabilize the signal).
- DC and high-gain AC amplifier (to improve signal clarity).
- Hewlett-Packard tape recorder (to store the data).
- Brush Recorder (to visualize the data).

3. Controlling Synaptic Transmission (Chemical Environment)

Artificial Seawater Solution: 55 mM Mg^{2+} and 11 mM Ca^{2+}

To modify synaptic transmission, they adjusted Mg^{2+} and Ca^{2+} levels:

High Mg^{2+} → Decreased neurotransmitter release.

High Ca^{2+} → Increased neurotransmitter release.

Why manipulate ions?

Magnesium (Mg^{2+}) blocks neurotransmitter release, so increasing it reduces synaptic transmission.

Calcium (Ca^{2+}) is needed for neurotransmitter release, so reducing it makes the neurons release fewer quanta.

4. Stimulating the Reflex & Measuring Synaptic Depression

Reflex Studied

- The gill-withdrawal reflex was triggered by stimulating the siphon (a part of Aplysia's body).
- The sensory neurons that detect touch activated the motor neurons, causing the gill to withdraw.

Repetitive Stimulation (Habituation Protocol)

- They stimulated the sensory neurons repeatedly (10-15 times).
- This led to habituation, where the motor neuron responses weakened over time.
- Inter-stimulus intervals (ISI):
  10 sec 30 sec 60 sec

5. Confirming monosynaptic connections

Before analysing synaptic depression, they had to confirm that the synaptic connections were direct (monosynaptic) rather than involving interneurons.

Criteria for Monosynaptic Connections:

- 1. One-to-one firing: The motor neuron responded with a single synaptic potential every time the sensory neuron fired.
- 2. Short and constant delay: The time between the sensory neuron firing and the motor neuron response was always the same (5-10 milliseconds).
- 3. Latency did not change under different conditions:
  - High Mg^{2+} levels (which block indirect pathways) did not change response timing.
  - Increasing neurotransmitter release (injecting TEA – tetraethylammonium chloride) did not change response timing.

6. Measuring Quantal Fluctuations

Quantal Analysis method

- Transmitter release happens in **quantal packets** (like droplets of a liquid).
- By **reducing neurotransmitter output** using high Mg^{2+} and low Ca^{2+}, they could see how **quantal release changed** over time

How they detected quanta

- Recording EPSPs (Excitatory Postsynaptic Potentials)
- EPSP size fluctuated in steps, showing distinct quantal release events.
- Sometimes, no neurotransmitter was released, leading to failure trials (no response).

Amplitude Histogram

- They plotted EPSP sizes and observed distinct peaks (each peak corresponding to an integer multiple of a single quantal event).

Computer Averaging

- Used a PDP8-E computer to analyze and confirm whether the recorded failures were real (not just noise).

7. Quantal Analysis & Data Processing

Mathematical Model Used: Poisson Distribution

*(The **probability of neurotransmitter release** was estimated using Poisson statistics.)*

- Formula Used: ***m=n×p***
- EPSP amplitude (EE) formula: ***E=m×q***

m = Mean number of quanta released
n = Total number of available quanta.
p = Probability of each quantum being released.
q = Mean size of a single quantal EPSP.

Three Ways to Estimate Quantal Content *(m)* and Size *(q)*

Failure Rate Analysis	• If neurotransmitter release follows a Poisson process, then the fraction of **failure trials** gives a measure of how often **zero** quanta were released.
Coefficient of Variation *(CV)* Method	• They calculated how much EPSP sizes **varied** over time to estimate ***q***.
Direct Amplitude Measurement	• Measuring **peak amplitudes** of EPSPs and counting multiples of the smallest unit.

Summary of Methods

- This method allowed them to conclude that short-term habituation is caused by a presynaptic reduction in the number of neurotransmitter quanta released, not a change in postsynaptic receptor sensitivity.

Removed the abdominal ganglia from Aplysia and kept them in an artificial seawater solution.

Recorded electrical activity from sensory and motor neurons using electrodes.

Manipulated ion concentrations (Mg^{2+}, Ca^{2+}) to control neurotransmitter release.

Repeatedly stimulated the sensory neurons to induce habituation of the gill-withdrawal reflex.

Confirmed the synapses were monosynaptic (direct connections between sensory and motor neurons).

Measured changes in neurotransmitter release using quantal analysis (amplitude histograms, failure analysis, Poisson distribution).