Assignment II Report: Simulation of a Monosynaptic Neuronal Network

1. Introduction

1.1 Background on Chronic Neuropathic Pain

Chronic neuropathic pain results from plastic changes in the peripheral and central nervous system. Unlike acute pain, which serves as a protective mechanism signaling tissue damage, neuropathic pain persists beyond the healing process and frequently manifests without any ongoing injury. Clinically, patients with neuropathic pain report symptoms such as hyperalgesia (exaggerated response to painful stimuli), allodynia (pain due to typically non-painful stimuli like touch), paresthesias (tingling or 'pins and needles' sensations), and spontaneous pain (pain without an apparent trigger). These symptoms are rooted in maladaptive changes that occur in both the peripheral and central nervous systems, disrupting the normal processing of pain signals.

1.2 The Peripheral and Central Pain Pathway

Pain signals originate in peripheral nociceptors and are conveyed by dorsal root ganglion (DRG) neurons, which are pseudo-unipolar sensory neurons, to the dorsal horn of the spinal cord. These neurons detect thermal, mechanical, or chemical stimuli and form glutamatergic synapses onto second-order neurons in the dorsal horn. The second-order neurons, many of which are also glutamatergic, relay the signal to higher brain centers via ascending pathways for further processing and perception.

The dorsal horn serves as a key integrative hub where both excitatory and inhibitory signals converge. GABAergic interneurons within this region provide crucial local inhibition, shaping the excitatory flow of nociceptive information. However, in chronic pain states, this inhibitory control is often diminished, resulting in central sensitization—a condition characterized by heightened excitability and responsiveness of dorsal horn neurons. This state is driven by persistent activation, disinhibition, and synaptic plasticity mechanisms such as long-term potentiation (LTP), which collectively enhance excitatory transmission and contribute to persistent, exaggerated pain signaling.

1.3 Acetylcholine and ACh Receptors (AChRs) in Pain Modulation

Acetylcholine (ACh) is a neuromodulator increasingly recognized for its role in pain processing. Its effects are mediated through **nicotinic acetylcholine receptors (nAChRs)**, which are ligand-gated ion channels composed of various subunit combinations. In our model, we have included specific receptor subtypes based on experimental relevance:

- **α7 AChRs** are included on the **glutamatergic neuron**, likely involved in modulating excitatory neurotransmission.
- **α3β4 and α4β2 AChRs** are placed on the **DRG neuron**, suggesting presynaptic modulation of sensory input.

These receptors are believed to mediate distinct effects on neuronal excitability:

- Activation of **α7 receptors** is commonly associated with reduced excitatory output, contributing to **anti-nociceptive effects**.
- **α3β4 and α4β2 receptors**, depending on their distribution and context, may regulate neurotransmitter release and influence the strength of synaptic signaling.

Thus, modulating AChR activity presents a promising therapeutic strategy for managing chronic pain.

1.4 Project Objectives

This project aims to simulate a **three-neuron spinal pain circuit** using the **NEURON simulation environment**, integrating:

- A **dorsal root ganglion (DRG) neuron** (sensory input).
- A **glutamatergic dorsal horn neuron** (excitatory relay).
- A **GABAergic interneuron** (inhibitory modulation).

Key objectives include:

- Constructing the morphological and electrophysiological models of each neuron, incorporating ion channel dynamics essential for excitability (**Na_v, K_v, Ca_v**).
- Implementing synaptic mechanisms reflecting biological realism: **AMPA**, **NMDA**, and **GABAA** receptors to represent excitatory and inhibitory transmission.
- Simulating the release of **acetylcholine (ACh)** from a cholinergic source and its effect through **α7**, **α3β4**, and **α4β2** AChRs located on DRG and glutamatergic neurons.

- Analyzing the resulting dynamics in terms of **membrane potentials**, **ionic currents (Na^+, K^+, Ca^{2+})**, and synaptic interactions to evaluate how cholinergic modulation influences network behavior under pain-processing conditions.

2. Methods

2.1 Software and Simulation Environment

All simulations were conducted using the **NEURON simulation environment**, which supports detailed biophysical modelling of individual neurons and complex networks. Custom .hoc and .mod files were written and executed to build neuron morphologies, implement ion channels, and configure synaptic interactions.

2.2 Neuron Model Construction

Literature and ModelDB Consultation

Neuron types and channel dynamics were adapted based on publicly available models on **ModelDB**. Morphological parameters were guided by literature and adjusted for simulation efficiency. Specific channels were implemented using .mod files adapted from established Hodgkin-Huxley-type mechanisms.

General Morphology

Each neuron was modeled with a **spherical soma** and **cylindrical axonal/dendritic segments**. While precise anatomical reconstructions were not used, the structure was designed to reflect biologically plausible compartmentalization for accurate current flow and synaptic localization.

Neuron 1: Dorsal Root Ganglion (DRG) Neuron

- **Morphology**: Spherical soma (30 µm diameter), multi-compartment axon (10 segments; each with appropriate passive and active properties).
- **Passive Properties**: Cm = 0.5 µF/cm^2, Ra = 150 Ω·cm, Rm = 10,000 Ω·cm^2.
- **Ion Channels**:
    - Fast Na^+: Hodgkin-Huxley type sodium channel
    - Delayed rectifier K^+
    - A-type K^+
    - L-type Ca^{2+}: Low threshold activation
- **AChRs**:
    - **α3β4 and α4β2** receptors inserted on the axon

- Modeled as ligand-gated channels with reversal potential ≈ 0 mV. Conductance values set in corresponding receptor definitions

Neuron 2: Glutamatergic Dorsal Horn Neuron

- **Morphology**: Spherical soma (25 µm diameter), axon segmented into 10 compartments
- **Passive Properties**: Cm = 1 µF/cm^2, Ra = 150 Ω·cm, Rm = 25,000 Ω·cm^2.
- **Ion Channels**:
  - Fast Na^+: Hodgkin-Huxley type sodium channel
  - Delayed rectifier K^+
  - A-type K^+
  - L-type Ca^{2+}: Low threshold activation
- **Synaptic Receptors**:
  - Receives **AMPA** and **NMDA** synapses from DRG neuron
  - Receives **GABAA** input from GABA interneuron
- **AChRs**:
  - **α7 nAChRs**, inserted on axon
  - Modeled as ligand-gated channels with rapid kinetics; reversal potential ≈ 0 mV

Neuron 3: GABAergic Interneuron

- **Morphology**: Spherical soma (20 µm diameter), 16 dendritic compartments (each 50 µm long, 1 µm diameter).
- **Passive Properties**: Cm = 1 µF/cm^2, Ra = 150 Ω·cm, Rm = 20,000 Ω·cm^2.
- **Ion Channels**:
  - Fast Na^+: Hodgkin-Huxley type sodium channel
  - Delayed rectifier K^+
  - A-type K^+
  - L-type Ca^{2+}: Low threshold activation
- **Synaptic Receptors**:
  - Receives **AMPA** and **NMDA** inputs from DRG and Glu neurons
  - Receives **GABAA** inhibition from itself (autoreceptors on soma)

2.3 Network Construction

- **Synaptic Connections**:
  - DRG → Glu neuron via AMPA/NMDA
  - DRG and Glu → GABA interneuron via AMPA/NMDA
  - GABA interneuron → DRG and Glu via GABAA
- **Neurotransmitter Release**:

    - Triggered by local depolarization; transmitter release modeled via pointer variables (e.g., T_rel) linked to receptor locations.
- **Synaptic Conductance**:
    - AMPA: 0.3 μS
    - NMDA: 0.05 μS
    - GABAA: 0.2 μS
    - AChR: 0.1 μS (varies by receptor subtype)

2.4 Simulation Protocol

- **Stimulation**:
    - The DRG neuron was stimulated using a **current clamp (IClamp)** applied to the soma of a cholinergic input neuron.
    - Parameters: Amplitude = 10 nA, Delay = 1 ms, Duration = 5 ms.
- **ACh Application / AChR Activation**:
    - ACh release was simulated through T_rel in the cholinergic neuron.
    - Receptor activation (α7, α3β4, α4β2) was driven by this release signal, which was propagated to corresponding receptors via pointer connections.
    - Conductances were preset in mod files; different levels of receptor activation were not explicitly varied in this model.
- **Recording**:
    - Recorded variables included:
        - **Membrane potential (Vm)** from the soma of all three neurons
        - **Sodium (INa)**, **potassium (IK)**, and **calcium (ICa)** currents from soma and axonal segments
- **Simulation Control**:
    - Total simulation time: tstop = 200 ms
    - Integration time step: dt = 0.025 ms
    - Initial membrane potential: v_init = -65 mV
    - Temperature: celsius = 37°C
- **Data Analysis**:
    - Outcome measures included:
        - **Firing frequency** and **spike timing**
        - **Action potential amplitude and width**
        - **EPSP characteristics** at synaptic targets
        - **Peak ionic currents** in soma and axon during activation

3. Results

3.1 Baseline Network Characterization

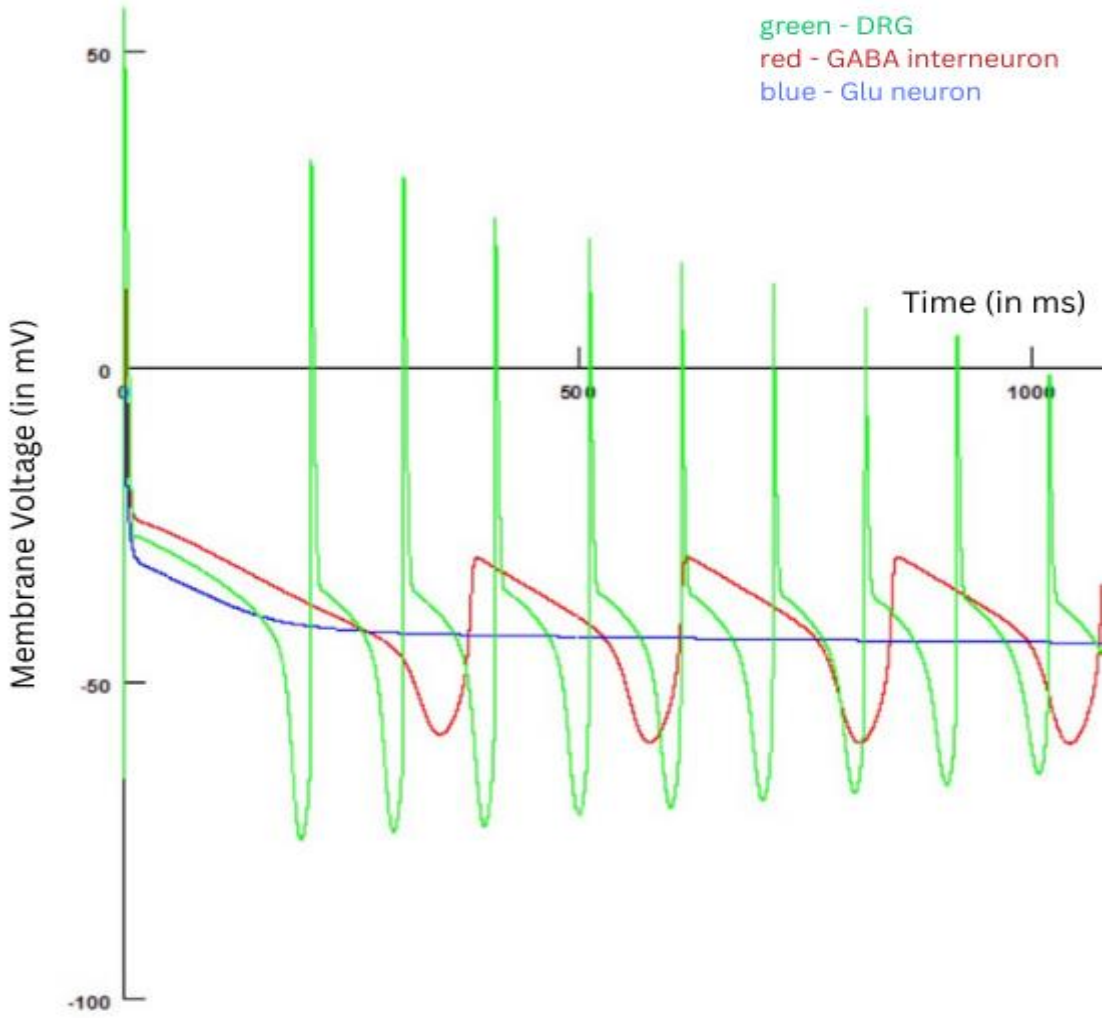

Figure 1: *Membrane voltage response in the DRG, GABAergic interneuron, and glutamatergic neuron.*

- Upon stimulation via IClamp, the **DRG neuron** (green) showed clear, repetitive **action potential firing**, confirming excitability.
- The **GABAergic interneuron** (red) displayed rhythmic firing, likely driven by input from the DRG and Glu neurons.
- The **glutamatergic neuron** (blue) showed subthreshold membrane depolarization without action potentials, suggesting inadequate synaptic excitation under current model settings.

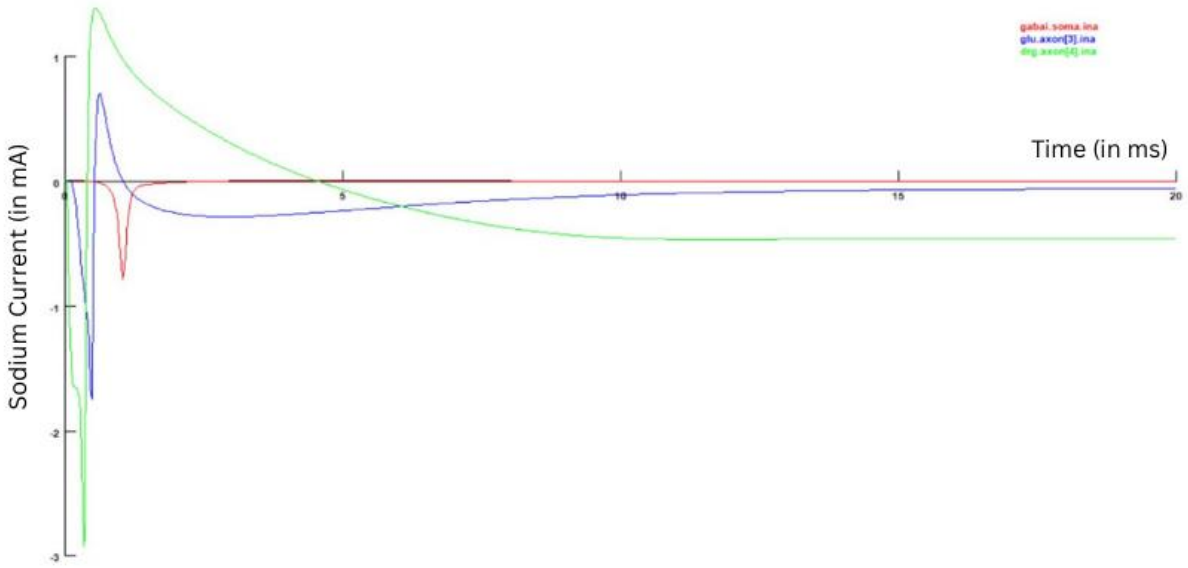

Figure 2: *Sodium current (INa) dynamics across the network.*

- The **DRG neuron** (green) exhibited the strongest INa transients, consistent with its robust spiking.
- The **GABAergic interneuron** (red) showed moderate sodium influx aligned with its firing.
- The **glutamatergic neuron** (blue) exhibited minimal sodium activity, consistent with lack of spiking.

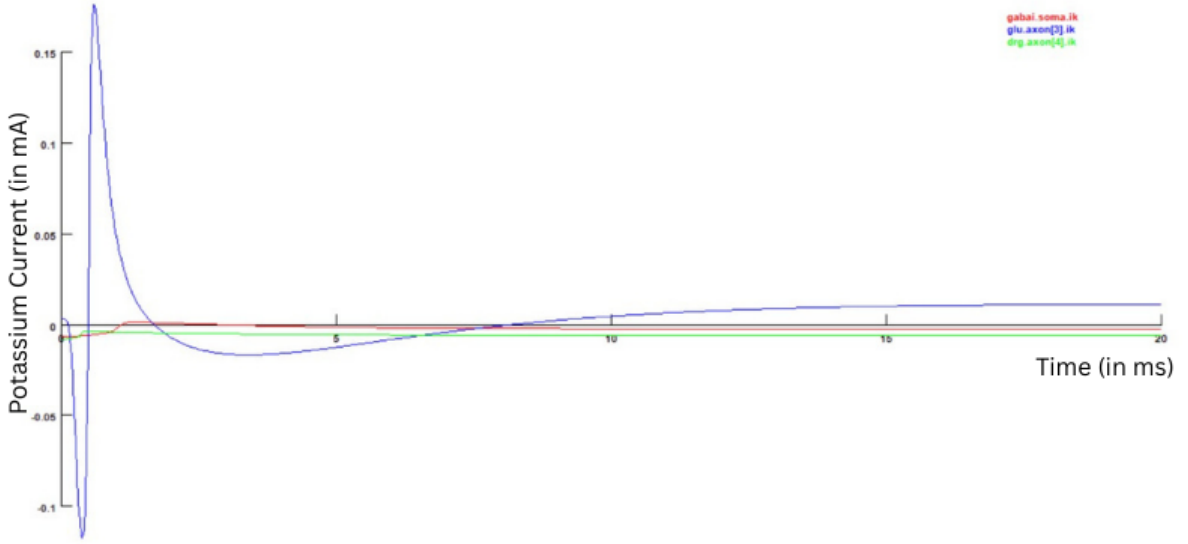

Figure 3: *Potassium current (IK) across neurons.*

- The DRG and GABA neurons both showed potassium efflux peaks after depolarization.

- The **GABA interneuron** (blue trace) displayed a biphasic IK response due to its bursting behavior.

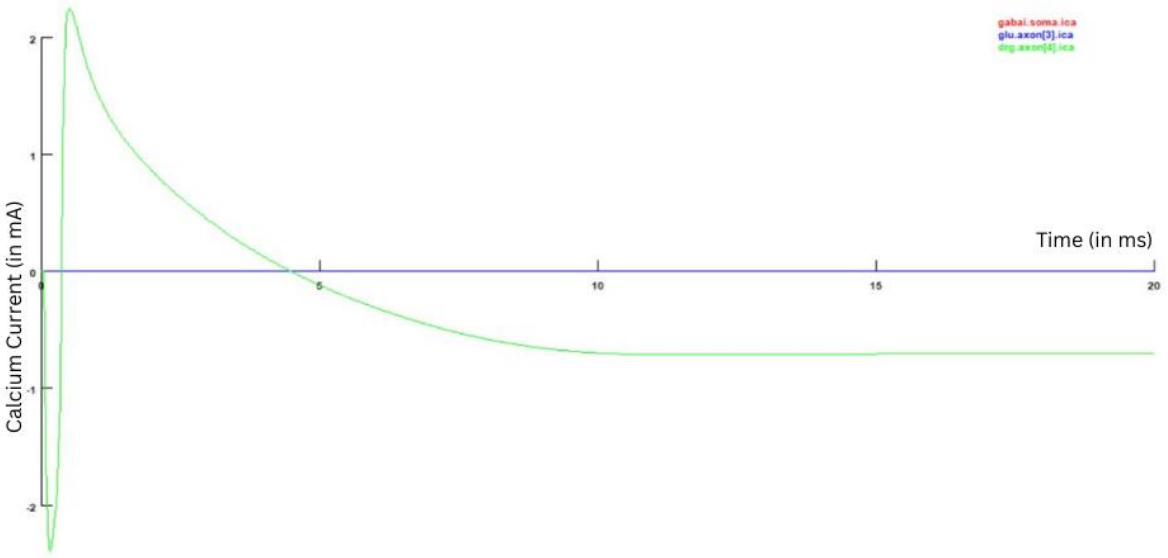

Figure 4: *Calcium current (ICa) in DRG, GABA, and Glu neurons.*

- Only the **DRG neuron** (green) showed prominent calcium current dynamics, peaking during spike onset.
- **Glu** and **GABA** neurons lacked noticeable ICa transients, indicating insufficient depolarization or improper tuning for Ca^{2+} channel activation.

3.2 Neurotransmitter Release and AChR Effects

- No clear evidence of neurotransmitter-driven **EPSPs** was observed in the postsynaptic Glu neuron.
- Absence of significant **calcium currents** in the Glu and GABA neurons suggests that synaptic release mechanisms may not have been sufficiently activated.
- This indicates a need for **further tuning** of:
    - Stimulus strength
    - Synaptic conductance
    - Postsynaptic ion channel properties

4. Limitations and Future Directions

4.1 Limitations:

- Synaptic release not strongly evident, likely due to insufficient presynaptic calcium activity.
- Simplified morphologies and uniform passive/active properties across neurons.
- Fixed ACh concentration, no dynamic ligand interaction modeled.

- Postsynaptic response limited due to likely low synaptic weights or receptor kinetics.

4.2 Future Work:

- Tune synaptic weights and firing thresholds for more reliable postsynaptic activity.
- Modify .mod files or receptor kinetics for greater ACh sensitivity.
- Introduce metabotropic AChRs or plasticity mechanisms (e.g., LTP) to simulate chronic pain adaptation.
- Use experimental data to guide conductance densities and receptor distributions.
- Explore paired-pulse and frequency-dependent synaptic dynamics.

5. Conclusion

This project aimed to model and simulate a simplified nociceptive circuit consisting of a DRG neuron, a spinal cord glutamatergic neuron, and a GABAergic interneuron using the NEURON environment. The baseline simulations confirmed that the DRG neuron could reliably fire action potentials in response to current injection, and that it successfully drove activity in the GABAergic interneuron. However, the glutamatergic neuron did not show substantial activation, likely due to insufficient synaptic input or conductance tuning.

Key ionic currents—including sodium, potassium, and calcium—were successfully recorded. Prominent currents were observed in the DRG neuron, while calcium currents were notably absent in the Glu and GABA neurons, limiting neurotransmitter release and synaptic propagation.

While acetylcholine receptor (AChR) subtypes such as **α3β4**, **α4β2**, and **α7** were included in the model, their functional influence on membrane excitability or synaptic dynamics was minimal under the current configuration. This suggests that AChR-mediated modulation requires further refinement in receptor kinetics or conductance to effectively alter circuit behavior.

In summary, the model lays a solid foundation for simulating ACh modulation in nociceptive signaling. With further calibration—particularly of calcium-dependent synaptic transmission—this system could become a valuable tool for exploring the roles of cholinergic signaling and receptor subtype-specific actions in chronic pain circuits.

6. References

- NEURON documentation: https://neuron.yale.edu/neuron/
- ModelDB: Senselab. (n.d.). *ModelDB*. Retrieved from https://senselab.med.yale.edu/ModelDB/
- NEURON mechanism and mod file tutorials: https://neuron.yale.edu/neuron/docs
- Carnevale, N. T., & Hines, M. L. (2006). *The NEURON Book*. Cambridge University Press.
- Albuquerque, E. X., Pereira, E. F., Alkondon, M., & Rogers, S. W. (2009). Mammalian nicotinic acetylcholine receptors: From structure to function. *Physiological Reviews, 89*(1), 73–120. https://doi.org/10.1152/physrev.00015.2008

7. Appendix

All relevant simulation files—including .hoc scripts for neuron models, .mod files for ion channels and receptors, and the driver.hoc control script—have been compiled into a ZIP folder and submitted along with this report. These files can be used to reproduce and extend the simulations described above.