

USER GUIDE

BRAINCELL 1.0.

*Brain cell *in silico**

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INTRODUCTION

Getting Started with BrainCell:

Welcome to the user manual for BrainCell!

BrainCell is a powerful simulation tool designed to evaluate the multi-scale morphology of astroglia and neurons, allowing you to create realistic multi-compartmental biophysical models of brain cells. With BrainCell, you can explore and analyse these models using the NEURON/Python computational environment, which provides a wide range of biophysical and cellular mechanisms. The primary objective of using BrainCell is to assist in the mechanistic interpretation of experimental observations in brain cells. This user manual will guide you through the features and functionality of BrainCell, enabling you to effectively leverage this tool and enhance your understanding of brain cell behaviour.

Key System and Software Requirements

To ensure a smooth experience with BrainCell, please ensure that your OS meets the following requirements:

1. ANACONDA/PYTHON (version 3.2 or later) - Download from <https://www.anaconda.com/download>
2. Neuron script C:\nrn\bin\nrnpyenv.sh uses the %APPDATA% variable, which is tied to the current user, so Anaconda must be installed only for the current user; otherwise, NEURON will not see it.
3. NEURON (version 7.2 or later) - Download from <https://neuron.yale.edu/neuron/download>

BrainCell Software: First-Time Installation Guide

Congratulations on choosing **BRAINCELL**, a powerful software for neural simulations. Follow the step-by-step instructions below to install BRAINCELL successfully on your Windows operating system. This guide will assist you in setting up the software and address common installation issues. Please note the following: **Ensure you have administrative privileges on your system.**

Unpacking and Running BrainCell

(For Users with NEURON and Anaconda Installed):

1. Start with BRAINCELL

- Download the **BRAINCELL** software package from <https://neuroalgebra.net/cloudpackage-v1.zip> with password on the Forum
- Extract the contents of the downloaded archive to a preferred location (e.g., c:\my\braincell).
- Ensure that all files and folders within the **BRAINCELL** directory are extracted.

2. Configuring File Permissions and Initiating NEURON Compilation

- For Windows 11 users, ensuring that any *.exe file in the NEURON directory, located explicitly at c:\nrn\bin\, operates with administrative rights is important. However, this step is optional for users with a version of Windows < 11. To achieve this, follow the steps outlined below:

3. Updating File Properties:

- Navigate to the NEURON directory c:\nrn\bin\ and locate the *.exe files. Adjust the properties of each *.exe file with administrative rights.

4. Run BrainCell

- Executing the init.Hoc / or init.bat. This file is in the directory path ...\\init.hoc.

5. Compiling NEURON .mod files:

- Once you have downloaded BrainCell, it will be ready to run. However, if you make any changes or add new mod files, you must activate build_mechs.bat to compile the NEURON *.mod files. This step is essential to ensure the proper functioning and integration of the NEURON modules into your system.

6. Running PS Scripts on Windows

- If your Windows configuration restricts the execution of batch (bat) files, you can opt to run PowerShell (ps1) files instead. Windows, by default, imposes restrictions on the execution of PS1 files. To overcome this limitation, follow the steps below:

- Open PowerShell in administrator mode. Run the following command:

Set-ExecutionPolicy -ExecutionPolicy Bypass -Scope CurrentUser

This command bypasses the execution policy for the current user, allowing PS1 files to run without restrictions.

This action is a one-time requirement; you will not need to repeat it in the future. After performing this step, you can effortlessly run PS1 files.

7. Running BrainCell

- Open the c:\my\braincell directory.
- Double-click on the "init.bat" file to execute it.
- This action should launch Neuron and open the **BRAINCELL** window.
- In the **BRAINCELL** window, you can select either "Astrocyte" or "Neuron" per your requirements.

Unpacking and Running BRAINCELL (For Users without NEURON and Anaconda Installed but with the previous version of Python installed):

If you do not have NEURON and Anaconda installed or are experiencing issues with BrainCell after their installation, please follow these step-by-step instructions to set up NEURON with Anaconda correctly:

1. Creating a New Windows User

- Press the Windows key + R simultaneously to open the "Run" dialogue box.
- Type "netplwiz" and click OK to open the User Accounts window.
- Select "Add..." to create a new user account.
- Create a local user account rather than a Microsoft account in Advanced mode.
- Complete the user creation process by following the on-screen instructions.

Skip New User Creation:

(Note: If you still need to install existing Python versions, Windows Subsystem for Linux (WSL), or Cygwin on your system, you can proceed without creating a new user account.)

2. Logging in as the New User

- Log out of your current Windows user account.
- Log in using the newly created user account credentials.

3. Installing Anaconda Python

- Download the Anaconda Python distribution for Windows from the official website.
- Run the installer executable file and follow the installation wizard's instructions.
- Select the default installation options unless you have specific requirements.
- Once the installation is complete, proceed to the next step.

4. Installing Neuron

- Download the Neuron software package.
- During installation, choose a destination folder other than the default location (e.g., c:\my\nrn).
- Follow the installation prompts and accept the default options unless instructed otherwise.
- Once Neuron is installed, proceed to the next step.

5. Unzipping BRAINCELL

- Download the **BRAINCELL** software package.
- Extract the contents of the downloaded archive to a preferred location (e.g., c:\my\braincell).
- Ensure that all files and folders within the **BRAINCELL** directory are extracted.

6. Adjusting File Permissions

- In Windows 10/11, navigate to the c:\my\braincell directory.
- Right-click on the "init.bat" file and select "Show more options" or "Properties".
- In the Properties window, scroll to the bottom and locate the "**Unblock**" checkbox.
- Check the "Unblock" box and click "**Apply**".
- Repeat the same process for the "build_mechs.bat" file in the same directory.
(This prevents Windows from blocking the execution of these batch files.)

7. Running BRAINCELL

- Open the c:\my\braincell directory.
- Double-click on the "init.bat" file to execute it.
- This action should launch Neuron and open the **BRAINCELL** window.
- In the **BRAINCELL** window, you can select either "Astrocyte" or "Neuron" per your requirements.

Troubleshooting Steps:

Initial Installation Attempt:

Follow the remaining steps of the installation process as outlined in the user manual. This includes installing Anaconda Python and Neuron, unpacking **BRAINCELL**, and editing the "init.bat" file as instructed. Adjust file permissions as mentioned in step 7 of the user manual.

Running BRAINCELL:

Execute the "init.bat" file from the "c:\my\braincell" directory to launch **BRAINCELL**.

If **BRAINCELL** starts successfully and you can select "Astrocyte" or "Neuron" within the application, you can use the software for neural simulations.

If you encounter any issues during the installation or when running **BRAINCELL** after following the troubleshooting steps, it is advisable to consult the user manual for further guidance. Additionally, our support team is available to assist you in resolving any technical difficulties you may encounter.

The strategy of building the model: summary

Creating a cell model using **BRAINCELL** can be a complex process, but here are some general instructions to get started:

1. **Basic 3D cell morphology.** Go to "NeuronMorpho" (<https://neuromorpho.org>) and search for the specific type of brain cell you want to model. Once you have found the cell 3D geometry, download it in the appropriate file format (such as SWC, OBJ or ZIP) in the home directory ...\\BrainCell\\Geometry\\ either ...\\Astrocyte or ...\\Neuron.
2. Open a **BRAINCELL** and import the 3D structure file.
3. Adjust the scale of the model to the appropriate size for your needs. This may involve resizing, repositioning, or rotating the model to match your desired dimensions.
4. Once you have created a basic 3D model of the brain cell, you can add nanostructures to the model using either an experiment or computer simulation.
5. If you want to add nanostructures to the astrocyte model using an experiment, you will need to use specialised software Astro in MATLAB to manipulate at the nanoscale level <https://neuroalgebra.net/astro.html>
6. Alternatively, you can use computer simulations to add nanostructures to the 3D cell. These simulations can help you to understand how the nanostructures interact with the brain cell and how they affect its function.
7. Once you have added the nanostructures to the model, you can use the 3D modelling software to visualise the changes and understand how they affect the overall structure and function of the brain cell.
8. Finally, you can refine and optimise the model as necessary to achieve your desired level of accuracy and detail.
9. Overall, creating a brain cell model using **BRAINCELL** is a complex process that requires specialised software and expertise in 3D modelling and nanoscale science. By following these instructions and utilising the appropriate tools and techniques, however, you can create a highly accurate and detailed brain cell model that can be used for a wide range of scientific and educational purposes.

The outlines of experimental data or approximations required to create a realistic cell model.

Here are the details:

1. It is preferable to have a 3D reconstructed tree of main cell processes that can be imported from <https://neuromorpho.org> in any format. Alternatively, an artificially generated cell arbour can be used, with the branching pattern and branch diameters representing the average (typical) cell from the population of interest.

2. Astrocyte nanostructures are essential, and a sample (20-50) of nanoscopic astroglial processes reconstructed using 3D (serial-section) EM is necessary. The sample should have rendered surface coordinates and will be used to obtain statistical properties of the ultrathin processes to be generated in the model.

3. Neuron nanostructures are also needed, and "BRAINCELL" can automatically generate synaptic spines with different distribution densities, geometries, and contacts with synapses. Synapses can be located both on the spines and directly on the dendrites. The user can select all parameters and control the spines' geometry complexity.

4. The average tissue volume fraction occupied by astroglia and neurons, as distributed radially from the soma to the cell edges, is also required. This data set can be obtained from two-photon excitation measurements *in situ* (or from published data).

5. It is necessary to have the mean membrane surface density and surface-to-volume fraction values, which can be obtained from 3D reconstructions of nanoscopic processes.

6. The characteristic I-V curve for the cell of interest, obtained through somatic patch-clamp with square-pulse current injections, is essential. Other available functional data, such as electrical responses to neurotransmitter uncaging or changes in extracellular ion and intracellular calcium wave speed, are optional but helpful.

GETTING STARTED

Installing and running BRAINCELL

Setting up and launching

The latest installation version can be downloaded from <https://neuroalgebra.net/>

On the website's front page (Fig. 1a), to download BrainCellNew, press the green key '**Clone or download**' and save **cloudpackage-v1.zip** with a password on the Forum (Fig. 1c) anywhere on your computer. Then, the archive must be opened, and its content saved on the Host computer (Windows/macOS), keeping the folder structure as described (Fig. 1b).

To start with **BRAINCELL**, the Host computer must have NEURON (7.0 or later) and Python 3. * Installed.

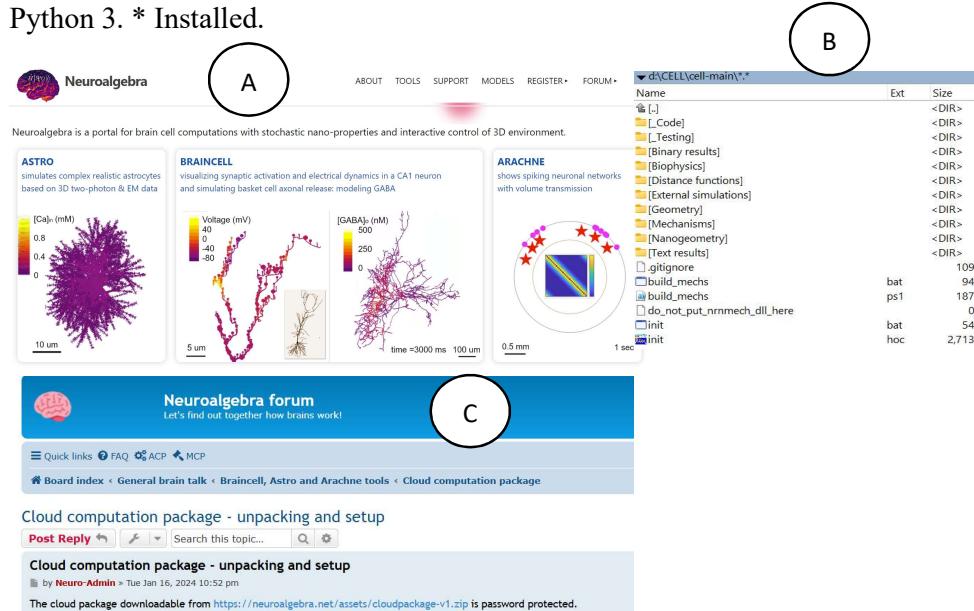


Figure 1. Screenshot of the BRAINCELL download GitHub page (a) and folder structure of BRAINCELL 1.0 on the Host computer (b).

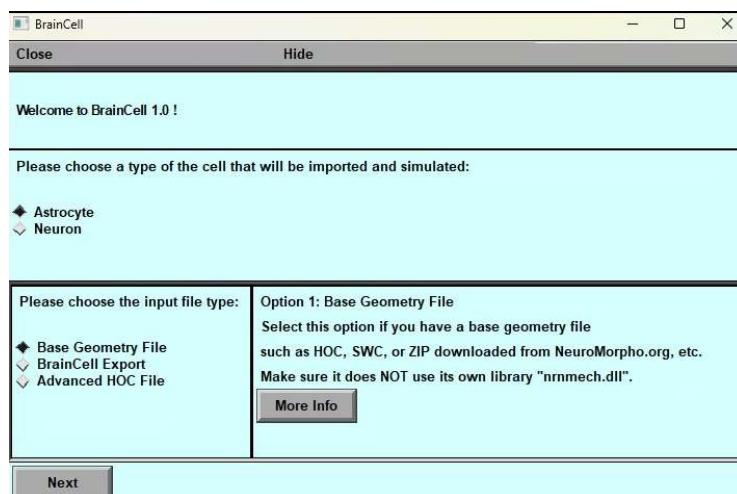


Figure 2. Introductory menu. Simulation Cell Configuration

The simulation interface's menu lets you choose between two types of cells: "Astrocyte" and "Neuron." Each cell type has two configuration options: "Base Geometry" or "BrainCell export" and "External Simulations".

1. Users can create and alter cells with variable 3D and nano shapes by selecting the " Base Geometry " configuration option.
2. Users can select " BrainCell export" from the drop-down menu to save time during simulation trials and upload a pre-existing cell with nanostructures. It's important to note that the shape of the loaded cell cannot be altered. This feature is helpful for those who prefer to avoid creating a new cell for each simulation run.
3. Select the "External Simulations" option if you have an advanced HOC file with biophysics or simulations, e.g., downloaded from ModelDB.science, and it does use its own library \"nrnmech.dll\".

Select one option for more information.

1. [Astro / Base.](#)
2. [Astro / Nano.](#)
3. [Neuron / Base.](#)
4. [Neuron / Nano.](#)
5. [External Simulations.](#)

Astro/ Base. Setting up and running BRAINCELL: Astrocyte configuration.

GENERATING COMPLETE ASTROCYTE MORPHOLOGY

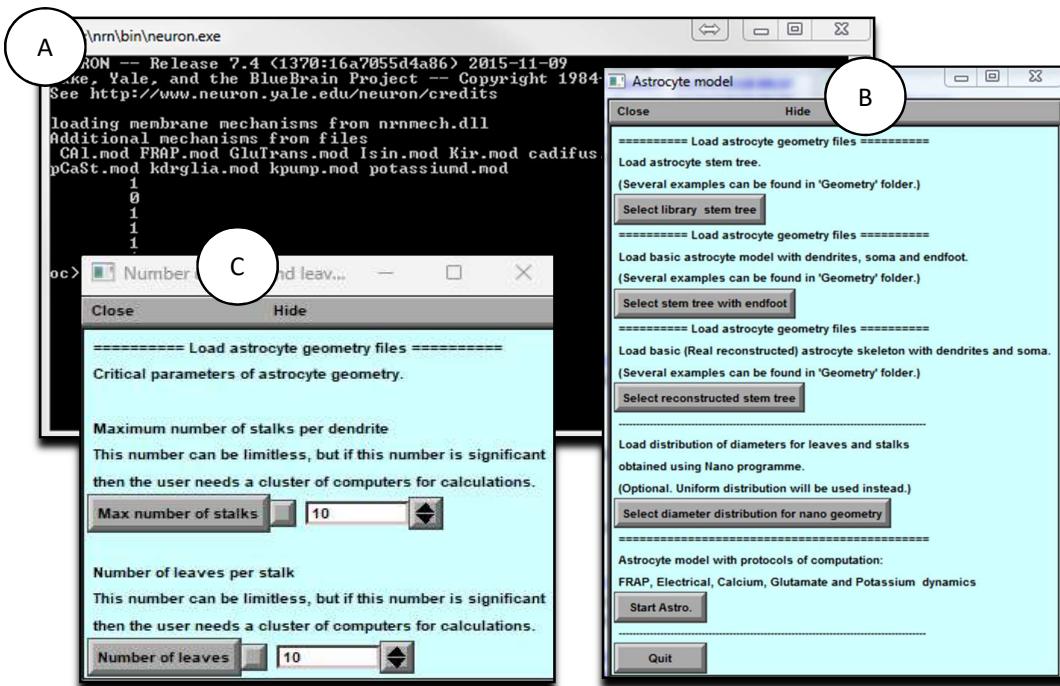


Figure 3. As the text details, control windows are initiated by launching NEURON in the BrainCell environment. The file init.hoc opens three windows: Command Console: Displays system messages and loading processes for simulation components (cmd.exe) (a), Astrocyte Model Panel: Users can select and load various pre-defined astrocyte geometry files for simulation. Options are available for basic structures or more complex dendritic and somatic forms. (stem tree, b), and a menu panel to set the density for higher orders of nanoscopic processes ('Leaf number' c) and the number of nanostructures per dendrites ('Max number of stalks', C).

Generating/downloading astrocyte stem tree

To design a new astrocyte model, the user has to define the basic structure of a dendritic tree using three different options:

Option 1: Click on "Select Library Stem Tree".

Choose to import 3D files in general zip format from the database <http://neuromorpho.com>. You can upload as many files as you like, but placing them in the directory.../Geometry/Astrocyte/New Style is recommended for convenience.

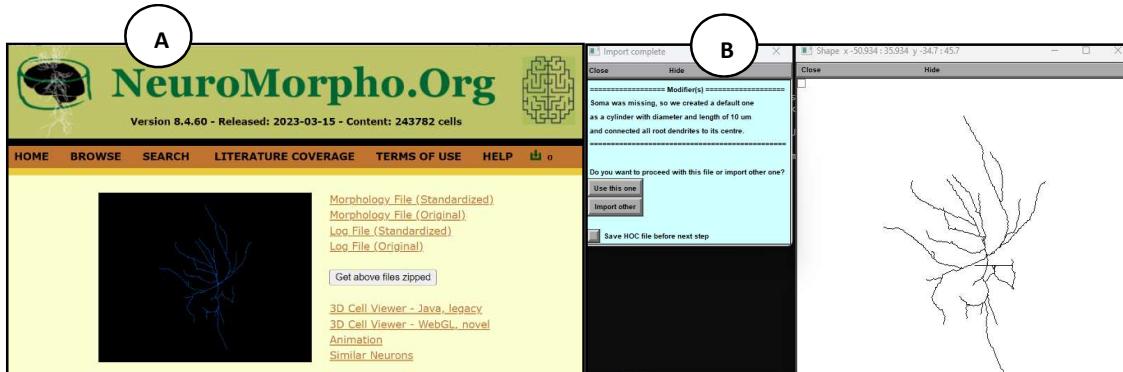


Figure 4. Importing 3D Cell Structure. A) The web page of NeuroMorpho displays the 3D shape of a cell. **B)** Operational BRAINCELL panels provide options for selecting astroglia morphology.

Alternatively, select a file from the in-house directory ...\\BrainCell\\Geometry\\ in SCW or HOC format.

To view a 3D structure of a file, please follow these steps:

- Select the desired file that contains a 3D structure, pressing “Select library stem tree”.
- You will be directed to a new window to view the 3D geometry of the selected file and choose the “Astrocyte/New Style” directory and file with 3D structure.
- If you like the structure, click the "Use this" button to proceed to the next step.
- If you do not like the structure, you can select another one by clicking the "Import another" button.
- Once you have chosen the desired 3D structure, you can proceed to the next option by clicking the appropriate “Use this” button.
- **OriginalDendrite** sets the number of branches (dendrites in NEURON terminology) on the stem tree. The database **NeuroMorpho.org** can be used as a guide to the ASTRO-compatible file format. Upon selection, a window panel displays the selected stem tree (**Fig. 5a**).

If you press "Use this" but change your mind later, don't worry; making a new selection is simple. Press the "Select Library Stem Tree" button again or choose "Select Stem Tree with Endfoot" from the options. Press the final "Start Astro" key if you are confident in your choice. Once you press it, you will not be able to go back.

Another option is to import the 3D geometry.

1. Editing the Endfoot Geometry:

A popup window will appear if you select the "Select Stem Tree with Endfoot" option. This window allows you to modify the geometry of the endfoot (refer to Fig. 5B) and provides a menu to set the morphology of the main and secondary endfoot branches and the local biophysical mechanisms.

2 'Select reconstructed stem tree' loads the 3D-reconstructed stem tree file. An example in *RealAstrocyteSkeleton1.hoc* (the directory .../Geometry) shows the reconstructed stem tree of the CA1 astrocyte using the Vaa3D software (Allen Institute, available from <http://www.alleninstitute.org/what-we-do/brain-science/research/products-tools/vaa3d/>).

This option also prompts an additional window panel (Fig. 17d), providing a setting for geometrical scaling and the centring of the astrocyte structure at the coordinate origin (to facilitate the positioning of selected cell compartments). The corresponding menu buttons thus include 'X-Y scale (pixel/ μ m)', 'Z scale (pixel μ m)', and 'X-Y shift (μ m)'. This window will disappear after any parameter change.

Note: Regardless of the case, a popup window directs you to the directory where the 3D file should be located. Make sure you have downloaded the required geometry file in advance using Neuromorphic.

3. Adding Nano Geometry to the Astrocytic Tree:

Once you have finalised the 3D geometry of the astrocyte, you have the option to incorporate nano geometry into the structure. To achieve this, you can use a file containing nano geometry that you have prepared using the Astro package. Select the "Select Diameter Distribution for Nano Geometry" option or press the "Start Astro" key. The BrainCell module will generate the astrocyte geometry randomly while ensuring it adheres to primary physiological constraints.

We recommend using the "Start Astro" key when initially acquainting yourself with the software program. This will help you familiarise yourself with its functionalities and capabilities.

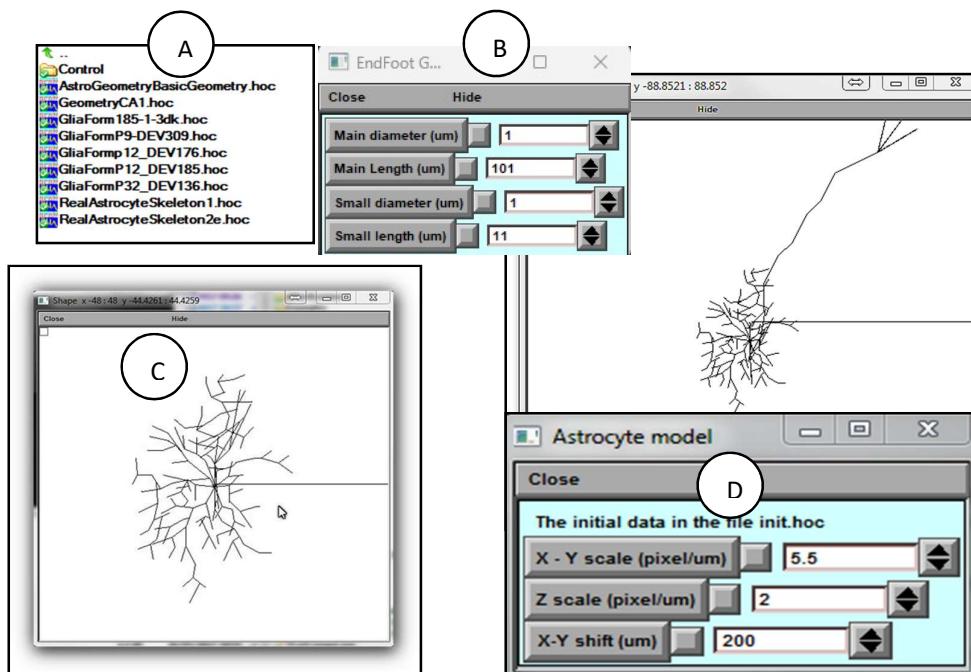
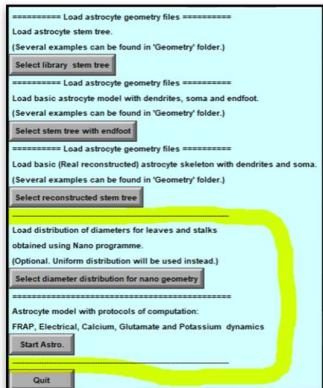


Figure 5. The BrainCell software's operational panels facilitate the creation and modification of astroglial morphology: A) File Navigation Panel: Displays a list of available hoc-files that define 3D astroglial shapes within the user's directory. B) EndFoot Geometry Panel: Allows for the design of astrocyte endfeet, with options to incorporate biophysical cell mechanisms. C) 3D Export Panel: Provides tools to export the astrocyte's 3D structure for further use or analysis. D) Morphology Transformation Panel: Enables scaling and shifting of the 3D shape, essential for detailed astroglial morphology adjustments.

NOTE: Before proceeding with further model design, the user must upload the cell stem tree geometry to the designated GEOMETRY directory.

Generating astroglial morphology on the nanoscale

The geometry of nanoscopic processes



Once the stem tree has been downloaded, the next stage is the nanostructure of the astrocyte. The user has two options on the popup window (see below), highlighted in yellow.

Option 1: To download the default nanostructure prepared in advance. Pressing the button '**Diameter distribution for nano-geometry**' prompts the user to download a file with the statistics of process diameters produced by the '**Nano (Geometry)**' module from the sampled 3D-reconstructed astroglial processes (see above). By default, this option downloads the file **testshape.dat_radii_dist.txt** (characterising astroglial processes in CA1 *stratum radiatum*). After that, the user presses the '**Start Astro**' button.

Option 2: Press the '**Start Astro**' button, in which case

BrainCell generates nanoscopic processes automatically using the built-in tools.

In both cases, the user can repeatedly adjust key morphometric features of the generated nanostructures. See further details in the chapter **Simulating Astrocyte Physiology**.

Populating astrocyte tree with nanoscopic processes

The '**Start Astro**' button prompts the main window panel '**Repertoire of computation**', critical to modelling complete astrocyte morphology, as described in the sections below.

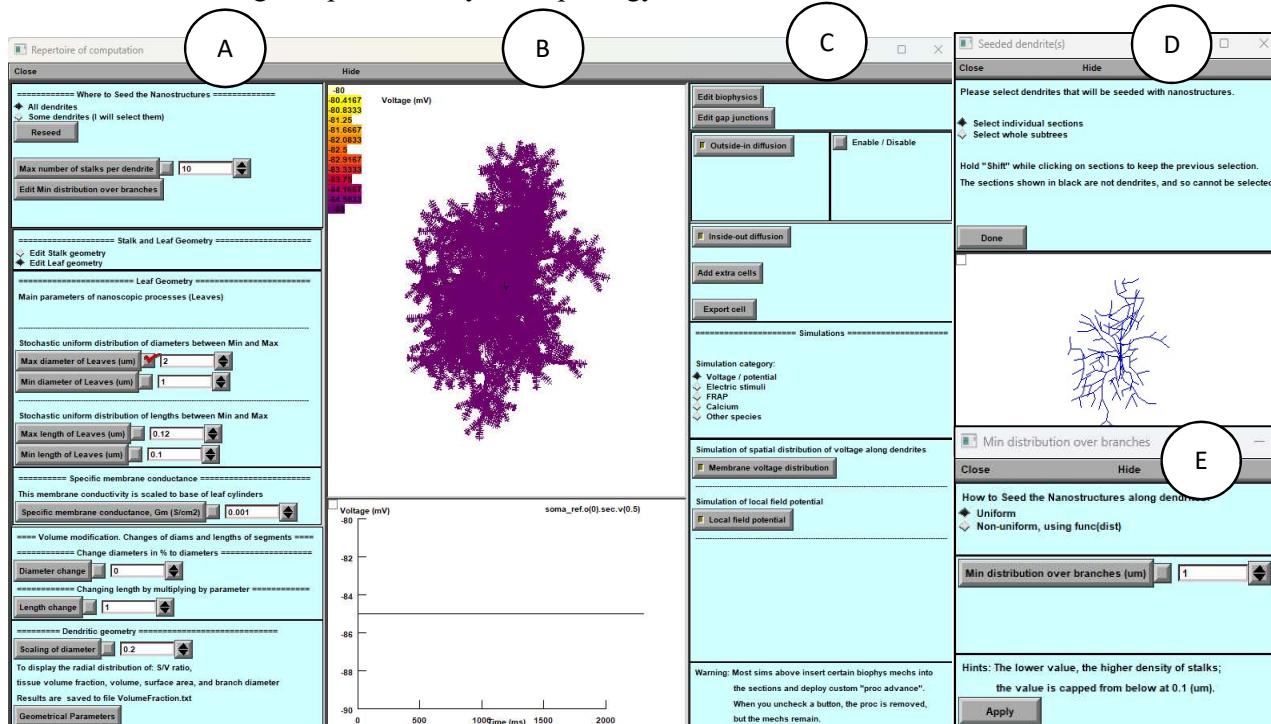


Figure 6. Astrocyte Main Window

- a) **Control Panel:** This panel provides an array of detailed settings for customising astrocyte geometry.
- b) **Simulated Variable:** The simulated variable is visually represented on the astrocyte morphology, with the top portion showing the mapping of variables (e.g., membrane voltage) and the bottom showcasing a selected digital output plot.

- c) **Biophysics, Stochastic, and Extracellular Sources Settings:** This section encompasses settings related to biophysical characteristics, stochastic elements, and extracellular sources. It's also where you configure computational scenarios with parameter adjustments.
- d) **Nanostructure Placement:** Users can define the location of nanostructures on the basic dendritic morphology, choosing to position them either throughout the entire astrocyte or exclusively on local dendrites.
- e) **Nanostructure Density Distribution:** This part allows you to set the density distribution of nanostructures, offering options for both uniform and non-uniform arrangements. These settings significantly impact the distribution and behaviour of nanostructures within the astrocyte.

One of the essential features for managing nano geometry is a key located in the upper left corner of the main window (Fig.6 A). This option enables the creation of nano geometry exclusively in a specific location within the dendritic tree. To utilise this feature, follow the steps outlined below:

- Switching from "All dendrites" to "Some dendrites" option: By toggling this option, you can restrict the nano geometry creation to specific dendrites.
- Initiating the "Reseed" process: Press the designated "Reseed" key to activate this process. This action will prompt an additional window to appear, providing further customisation options for the placement of the nano geometry.
- Specifying the desired location: In the newly opened window, you can specify where to add the nano geometry. This level of control allows you to target specific areas within the dendritic tree.
- Selecting dendrites: In the window, select the desired dendrites. This step involves identifying the dendrites where you intend to generate an astrocyte with localised nanostructures.
- Finalizing the process: After selecting the desired dendrites, press the "Done" key to generate the astrocyte with the specified nanostructures localised to the chosen dendrites.

NOTE: This option will increase computational requirements, mainly when focusing on very localised processes within the astrocyte. It is beneficial for users who are interested in studying fine-grained details within the astrocyte's local environment.

Panel '**Leaf Geometry**' (Fig. 6a, top) provides an option to set up the distribution of cylindrical compartments (leaves) of nanoscopic processes as evenly random (with lower and upper limits) when the experimental statistics on 3D reconstructed processes are not available.

NOTE: This section will be ignored when the latter has already been loaded (see previous section).

Panel '**Stalk Geometry**' (Fig. 6a, middle) sets upper and lower limits for the uniform distribution of transitional cylinders of nano geometry. These parameters determine how densely the tissue will be filled with nanoscopic astroglial processes.

The panel 'Specific membrane conductance' sets this value at the button 'Gm (mS/cm²)', which considers all exposed surfaces of the cylindrical compartments. The current's resting potential is -85 mV. This parameter is defined on the built-in NEURON panel' Distributed mechanism.'

Panel '**Dendritic Geometry**' (Fig. 6a, bottom) currently includes '**Branch diameter scaling**', which sets the scaling coefficient for the stem tree branch diameters as a function of distance from the soma, according to the average experimental trend. The empirically established formula for the branch diameter d is $d \sim (S(r+I))^{-1/2}$ where '**scalingDiam**' value S and r is the distance to the soma.

Customizing Cell Geometry

You can access advanced settings for more profound alterations to the cell's geometry. By selecting "Edit Biophysics" and "Edit Morphology," you can modify the morphology of any cell component based on the distance from the soma, following any mathematical rules of your choice. This level of customisation empowers you to craft intricate and tailored cell structures to suit your specific research or simulation needs.

NOTE: This panel has to be ignored if a 3D-reconstructed stem tree has been uploaded.

Tissue-filling properties of astroglial morphology

The tissue volume-filling properties and the surface-to-volume ratios of the nanoscopic processes will be determined by the shapes and the effective density of simulated nanoscopic processes, as described in the previous section. Tissue volume filling and other geometry features of the model can be monitored by pressing the '**Geometrical parameters**' key (Fig. 6): this opens several window panels displaying various parameters of the modelled cell geometry (Fig. 7). The displayed data are automatically saved to the file ...\\neuronSims\\Text results**VolumFraction.txt**.

Below are the key features of the main window:

1. **Nano geometry modification**: Users can add and modify spines' nano geometry to study the impact of structural changes on neuron behaviour.
2. **Biophysical mechanisms addition**: The tool enables users to add and change various biophysical mechanisms to neurons to investigate their impact on neuron behaviour.
3. **Synapse distribution**: The tool facilitates the addition and distribution of different types of synapses on the dendritic tree, enabling the study of the neuron's connectivity and behaviour.
4. **Simulation models**: Users can choose various modes to simulate the neuron's behaviour accurately. The simulation models include voltage clamp, current clamp, and dynamic clamp.

The neuron simulation tool's main window provides the necessary features to simulate and analyse neuron behaviour effectively.

"Geometric parameters" button

The "Geometric parameters" button allows you to calculate graphs of the main geometric parameters of the cell.

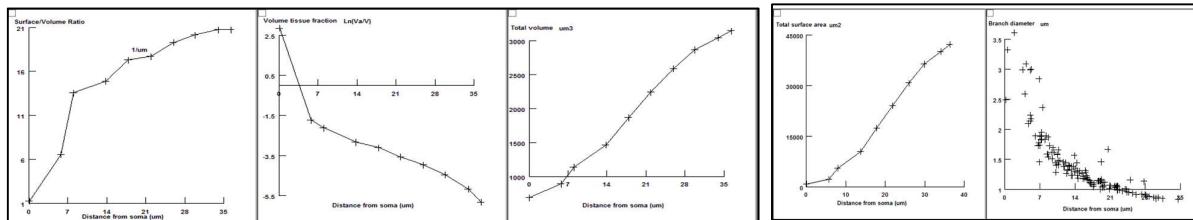


Figure 7. Window panels read the volumetric characteristics for modelled astroglia (launched by the 'Geometrical parameters' button).

From the top left: surface-to-volume ratio distribution, tissue volume fraction, total cell volume (cumulative value with the distance from the soma), total cell surface area, and diameters of primary processes.

The morphometric characteristics of the simulated astrocyte (Fig. 7) are to be compared with the corresponding empirical data obtained using 3D EM reconstructions and two-photon excitation imaging data for the astroglia of interest. The user can evaluate the mismatch and adjust the density of nanoscopic processes (using '**Stalk Geometry**' and '**Dendritic Geometry**' options where relevant; Fig. 6) correspondingly until an acceptable match is produced. The windows depicting critical geometrical parameters (Fig. 7) can be viewed anytime during modelling.

At the end of this stage, the modelled astroglial morphology is complete (see 'FRAP experiments' below for further subtle morphological adjustments). The user can begin to simulate various astroglia functions while implementing various membrane and intracellular biophysical mechanisms, as briefly explained in the following sections:

1. **Nano geometry modification**: Users can add and modify spines' nano geometry to study the impact of structural changes on neuron behaviour.

2. **Biophysical mechanisms addition**: The tool enables users to add and change various biophysical mechanisms to neurons to investigate their impact on neuron behaviour.

3. **Synapse distribution**: The tool facilitates the addition and distribution of different types of synapses on the dendritic tree, enabling the study of the neuron's connectivity and behaviour.

4. **Simulation models**: Users can choose various modes to simulate the neuron's behaviour accurately. The simulation models include voltage clamp, current clamp, and dynamic clamp.

The simulation tool's main window provides the necessary features to simulate and analyse behaviour effectively.

Nano Astro. Download previously created astrocyte morphology.

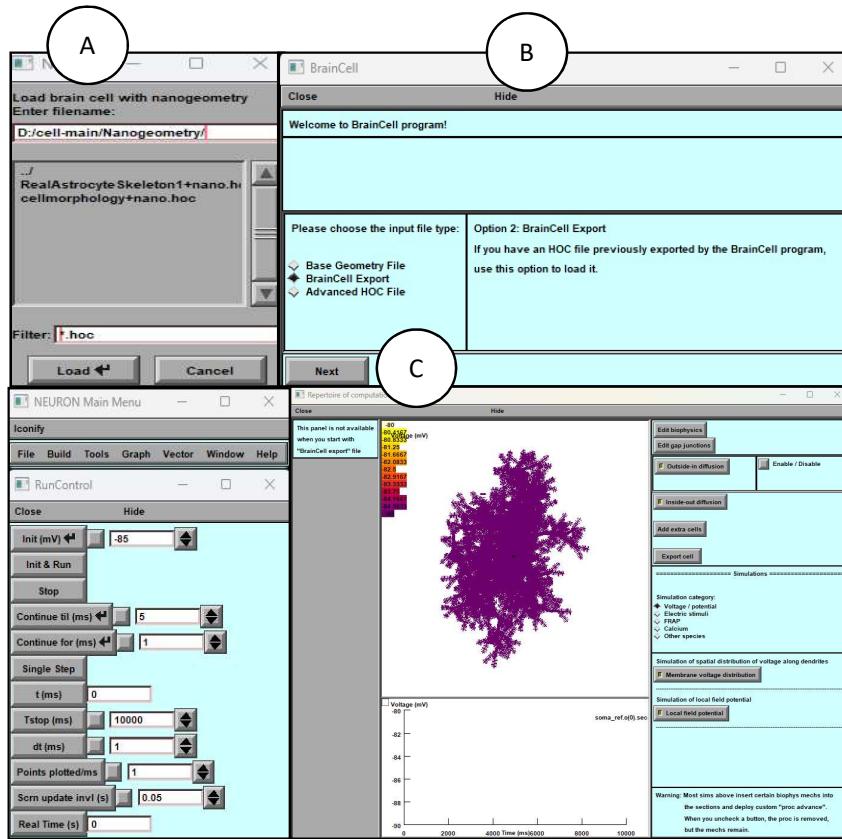


Figure 8. Operational Window Panels for Astrogli Morphology of Pre-existing 3D Astrocyte Structure

A) Download Panel: Allows access to the previously prepared 3D astrocyte structure.

Visual: Display a simple icon or representation of downloading data.

B) Roadmap Panel: Provides options to choose between a new or pre-existing astrocyte structure.

Visual: Show a roadmap-like graphic with two branches, one leading to a new astrocyte (**Base**) and the other to the pre-existing structure (**Nano**).

C) Schematic Illustration Panel: Illustrates the full 3D and nanostructure of the pre-existing astrocyte.

Visual: Show a clear and concise schematic diagram of the astrocyte's 3D and nanostructure.

To download an astrocyte morphology using "Astro + Nano" (Fig.8 B), pop up the panel and follow the steps to open the NEURON Basic Panel. It will help you locate the previously prepared astrocyte with Nanostructure.

Select the astrocyte with nanostructure to proceed with simulation and biophysical mechanisms management. This will take you to a new option for simulation and management.

At this stage, you can simulate and manage the biophysical mechanisms of the selected astrocyte with Nanostructure. Please note that you cannot change the geometry at this stage.

Below are the key features of the main window:

5. **Nano geometry modification**: Users can add and modify spines' nano geometry to study the impact of structural changes on neuron behaviour.

6. **Biophysical mechanisms addition**: The tool enables users to add and change various biophysical mechanisms to neurons to investigate their impact on neuron behaviour.

7. **Edit Gap Junction**: The tool facilitates the addition and distribution of different types of gap junction on the cell, enabling the study of the cell's connectivity and behaviour.

8. **Synapse distribution**: The tool facilitates the addition and distribution of different types of synapses on the dendritic tree, enabling the study of the neuron's connectivity and behaviour.

9. **Simulation models**: Users can choose various modes to simulate the neuron's behaviour accurately. The simulation models include voltage clamp, current clamp, and dynamic clamp.

The neuron simulation tool's main window provides the necessary features to simulate and analyse neuron behaviour effectively.

Neuron / Base. Setting up and running BRAINCELL: Neuron module.

IMPORTING AND GENERATING COMPLETE NEURON MORPHOLOGY

In this section, we will describe the **BRAINCELL** part specifically designed for constructing a 3D geometry model of a neuron. Please note that this part is different from the one used for astrocytes. When you load a 3D neuron, you will be presented with two windows that will allow you to determine the critical features of the neuron's structure. The first window will enable you to set the maximum number of spines on the dendrite (Fig.9A and B). These windows are critical and can be changed in the future, along with the geometry of the spines.

To accurately represent all parts of a cell, you can use the window shown in Figure 9C. It helps you identify the neuron's soma, dendrites, and axon. If the axon is not defined, the program can generate a basic one, or you can select one from a different section. You can edit the geometrical parameters later. Ensure each component has a unique name; otherwise, the software will show an error message. To create a 3D neuron, select your preferred configuration and click OK. The program will generate the form you have chosen, which you can use to assemble other neuron models or try another form from the database.

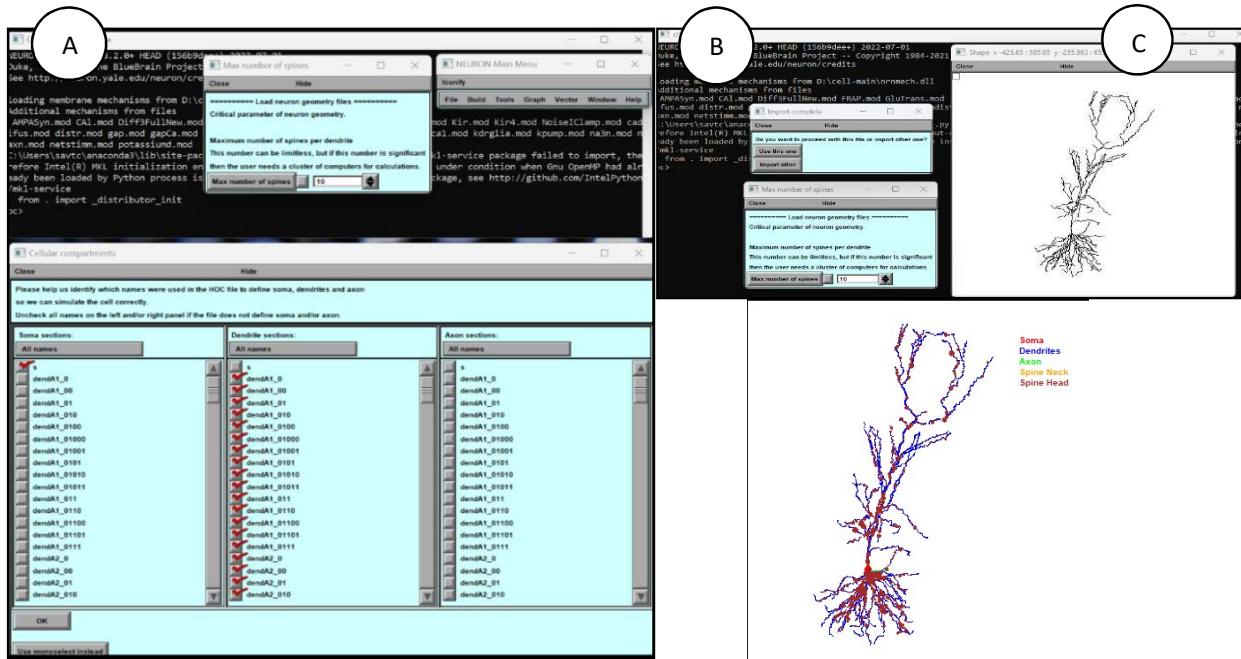


Figure 9. Import, Selection, and Final Shape of the 3D Structure of a Nerve Cell. A) Cell Structure Definition Panel: This crucial panel empowers users to define the cell's soma, dendrites, and axon. Future updates allow users to customize geometry vocabulary and organise dendrites into subgroups. **B) Cell Shape Selection Panel:** This panel provides options to select the desired final shape of the cell. **C) Spine Density Control Panel:** This panel adjusts the maximum number of spines per dendrite. It should be noted that larger dendrites generally exhibit lower spine density. In future iterations, users will have the ability to modify spine density.

Fig. 9 illustrates the process of importing, selecting and shaping a 3D structure of a nerve cell. Panels demonstrate:

- the import process, where the user can identify the soma, dendrite or axon and determine the maximum number of spines on the longest dendrite,
- displays the selected structure, enabling the user to accept or import another structure for viewing.

This figure provides a visual representation of the crucial steps involved in the process of constructing a 3D model of a nerve cell. If the user decides to try another neuron morphology, the procedure must be repeated, as previously described. If the user decides to stop at this one, a new window will appear by pressing the "**Use this one**" button.

Using **BrainCell**, users can customise the geometry and morphology of neurons by selecting the desired parameters and pressing the "**use this Neuron**" button. This will open the main window, providing access to various functions that can improve the cell's geometry, visualise any changes, and begin incorporating various biophysical mechanisms with different spatial distributions and stochastic properties.

Navigating Neuron Simulation: Exploring Main Windows for Enhanced Analysis

Our software provides users with a powerful tool to customise and analyse neurons' geometry and biophysical properties, all within an easy-to-use interface.

It is important to note that when setting up biophysical mechanisms, users can combine different parts of cells, such as the neck and heads of spines, which can be referred to as a single spine. Alternatively, users can divide dendrites, for example, into proximal and distal dendrites.

In Fig.10's main window, users can adjust cell geometry and test biophysical mechanisms to better understand neuron actions.

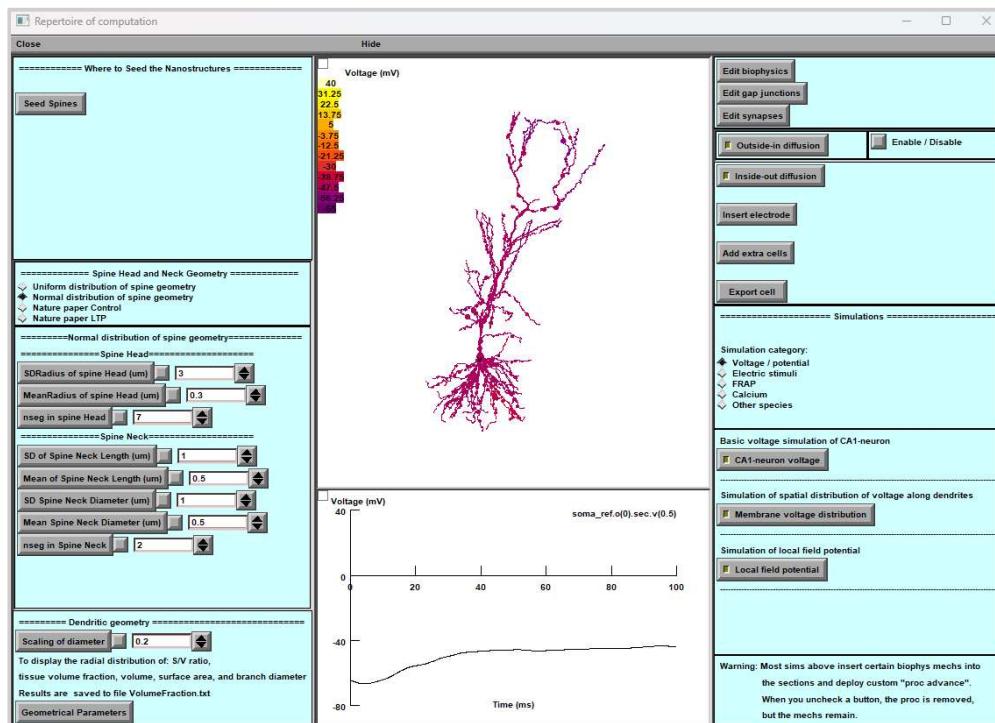


Figure 10. Main window for neuron simulation. The tool's main window provides several options to simulate neuron behaviour effectively.

Below are the key features of the main window:

- **Nano geometry modification:** “**Seed Spines**”: Users can add and modify spines' nano geometry to study the impact of structural changes on neuron behaviour.
- **Edit Gap Junction.** The Gap Junction editing feature enables users to customise their simulations by adding new Gaps or adjusting existing ones. This function defines and manipulates the various characteristics and distributions of different Gap Junctions to match specific research needs.
- **Biophysical mechanisms addition:** The tool enables users to add and change various biophysical mechanisms to neurons to investigate their impact on neuron behaviour.
- **Synapse distribution:** The tool facilitates the addition and distribution of different types of synapses on the dendritic tree, enabling the study of the neuron's connectivity and behaviour.
- **Edit extracellular source.** The "Edit Extracellular Sources" function within BrainCell allows you to customize the simulated environment in which your cells operate. Here's how to use it: Manages ions: Controls the types and concentrations of ions present

outside of your simulated cells. Flexibility: Gives you precise control over the cellular environment, enabling you to tailor it to your research needs.

- **Simulation models:** Users can choose various modes to accurately simulate the neuron's behaviour. The simulation models include voltage clamp, current clamp, and dynamic clamp.

The neuron simulation tool's main window provides the necessary features to effectively simulate and analyse neuron behaviour.

Customizing Neuronal Connectivity: Harnessing BrainCell's Spine Set Configuration.

Configuring Spine Sets

BrainCell provides powerful tools to customise the spines on your neuron models. Spines are tiny protrusions on neurons that form connections (synapses) with other neurons. With BrainCell, you can:

- **Create different spine distributions:** Model unique spine patterns for different parts of your neuron.
- **Customize spine properties:** Adjust the size, shape, electrical characteristics, and even the randomness of spines within a set.
- **Precision control:** Fine-tune your simulations by editing each spine set individually.

Using the Spine Sets Feature

1. **Access:** Find the "Spine Sets" option within BrainCell (the exact location might vary based on your version).
2. **Primary Panel:**
 - **Add a Spine Set:** Click "Add Spine Set" to create a new group of spines with specific characteristics.
 - **Remove a Spine Set:** Select an existing spine set and click "Remove Spine Set".
1. **Auxiliary Panel:**
 - **Visualize Spines:** This panel displays your neuron model, showing you where the spines from different sets are located.

Additional Notes

- For detailed instructions on editing the properties of individual spines within a set, refer to the "Editing Spines" section of the BrainCell manual.
- Changing spine configurations can have a significant effect on your neuron simulations.

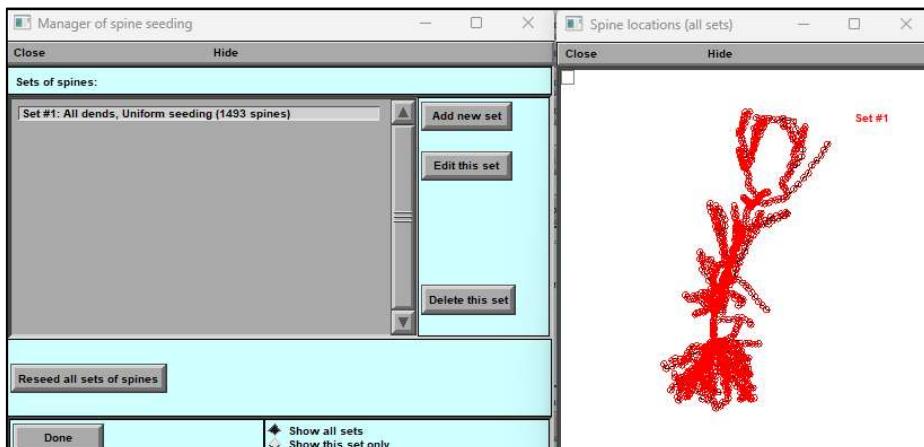


Figure 11a. The panel displayed is used to manage spine seeding.

The left panel, titled "Manager of spine seeding," lists the sets of spines and provides options to add, edit, or delete a set of spines. It also includes a "Reseed all sets of spines" button for resetting. At the bottom, "Show all sets" and "Show this set only" are available, along with a "Done" button to conclude the session.

The right panel, titled "Spine Locations (all sets)," visually represents the locations of spines on dendritic structures, with the current set highlighted.

Users can add and modify spines' nano geometry to study the impact of structural changes on neuron behaviour.

The editing of spine locations.

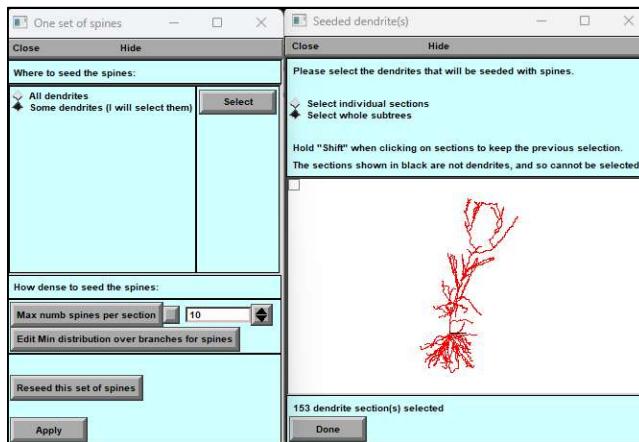


Figure 11b. The image shows two panels for the distribution of spine seeds.

Left Panel - One Set of Spines:

Options to select dendrites for spine seeding: either all dendrites or specific ones.

Ability to set the density of spines, including the maximum number of spines per section.

A button to modify the minimum distribution over branches for spines.

A button to reseed spines for the current set.

An '*Apply*' button to execute the changes.

Right Panel - Seeded Dendrite(s):

Instructions on selecting dendrites or subsections for spine placement, with a method to preserve previous selections.

A visual representation of a neuronal structure with highlighted areas where spines will be seeded.

Information on the number of dendritic sections selected.

A 'Done' button to finalise the selection process.

The "Where to Seed the Nanostructures" tab (Fig. 11) allows users to distribute the spines on specific dendrites and across the cell.

The user can distribute the spines on individual dendrites by clicking (using the mouse) on a specific dendrite (+Shift for several dendrites) on a panel presenting the neuron's 3D structure.

If you are happy with your chosen distribution, click the "Done" button. You may, of course, adjust the distribution of synapses at any time.

The spine, head and neck geometry. Location over dendritic tree.

This part explains how to use the tool to analyse the geometry distribution of dendritic spines (Fig.12). The panel provides two distribution options, regular and uniform, allowing the user to set numerical parameters for each distribution via a window upon selection. The tool also considers the minimum distance between synapses in a dendritic tree as an essential parameter for synapse distribution.

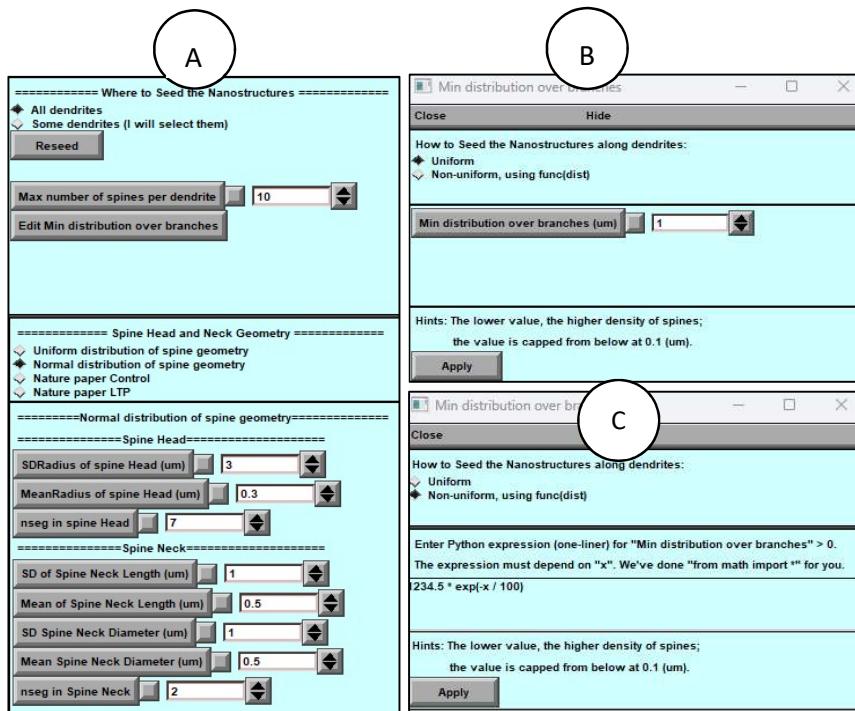


Figure 12. Spine Geometry Modification Panel

- Main Panel of Spine Geometry:** This central panel provides options and controls for modifying the geometry of spines within the dendritic tree.
- Panel for Uniform Distribution:** This sub-panel is dedicated to achieving a uniform distribution of spines across the dendritic tree and offers precise control over their arrangement.
- Panel for Non-Uniform Distribution:** In this sub-panel, spines are distributed non-uniformly using a user-defined formula, allowing for customised and intricate spine arrangements.

Selecting a Distribution:

To use the tool, the user must first select a distribution type. Two options are available: regular and uniform. The user can select a distribution type by clicking on the respective button on the panel (Fig.12). Once the user has selected a distribution type, they can set numerical parameters for each distribution via a window that appears upon selection.

Using Pre-Established Distribution Types:

The tool provides pre-established distribution types of spine geometry to make it more convenient for the user. These pre-established distribution types have been published in Tønnesen, J., Katona, G., Rózsa, B. et al. Spine neck plasticity regulates the compartmentalisation of synapses. Nat Neurosci 17, 678–685 (2014). <https://doi.org/10.1038/nn.3682>. To use these pre-established distribution types, the user can click on the respective button on the panel. This will provide the user with experiment-measured parameters for the selected distribution type.

Adjusting Spine Complexity:

Another crucial factor in determining spines is their spatial complexity (number of segments), which affects calculation speed. The number of segments can be adjusted with a minimum of two and no maximum limit. The user can adjust the complexity parameter using the panel slider.

Defining Synapse Distance:

The tool also considers the distance between synapses within a dendritic tree, which is crucial for synapse distribution. You can control this distance in uniform and non-uniform ways, as shown in Fig. 12. B and C. The distance parameter is inherently stochastic, which means the tool incorporates an element of chance in its calculations. Users can adjust the minimum distance between synapses using the provided panels. For the non-uniform distribution of synapse distances in the spatial domain, users can create a custom Python-format formula in a single line and apply it by clicking the "Apply" button. This feature allows for precise control over the spatial arrangement of synapses within the dendritic tree, accommodating random and user-defined patterns.

In conclusion, this tool allows users to analyse the geometry distribution of dendritic spines. The tool offers two distribution options, regular and uniform, which the user can select. The user can set numerical parameters for each distribution via a window upon selection. The tool also considers the minimum distance between synapses in a dendritic tree as an essential parameter for synapse distribution. Users can adjust the minimum distance between synapses and the complexity parameter to suit their requirements.

“Nano Neuron”. Download the previously created neuron model.

The combination “Neuron + Nano” creates a new panel. Once the panel appears, you can proceed with the following steps and open the NEURON Basic Panel to locate the previously prepared Neuron with Nanostructure. To proceed with the simulation and management of biophysical mechanisms, select the neuron with Nanostructure in hoc-file. This will take you to a new option for simulation and management (see Fig.13).

Note: At this stage, you cannot modify the geometry.

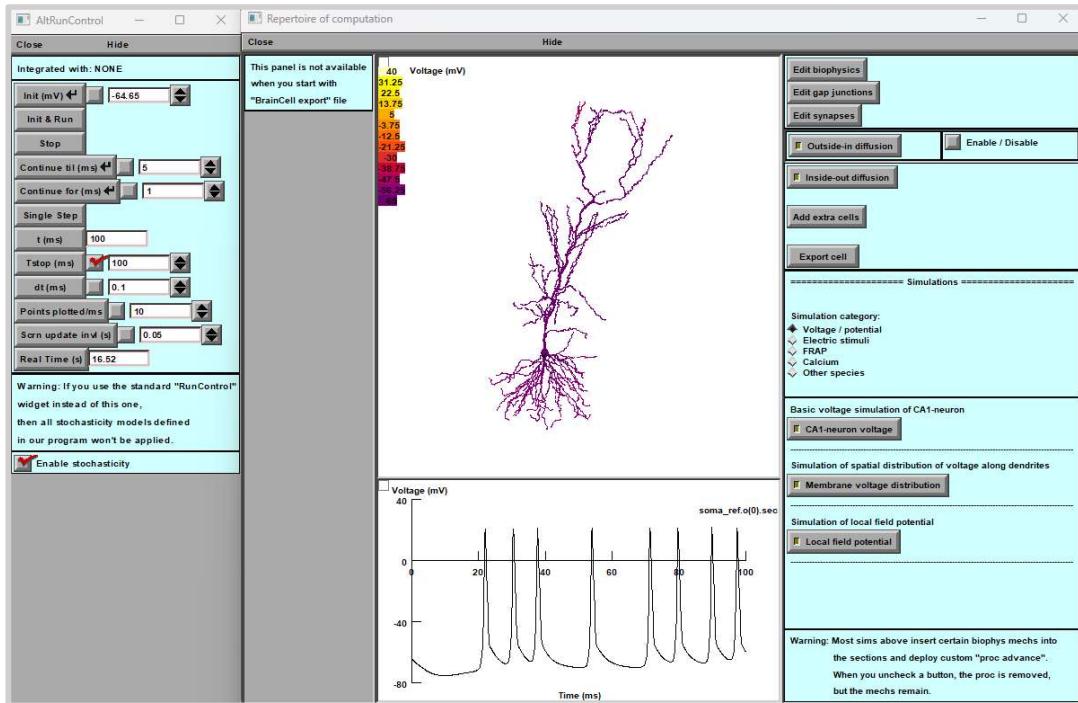


Figure 13: Left: The "Alternative Run Control" panel is designed to initialise and run simulations with specific settings like initial membrane voltage, run time, and integration step size, including options for continuous or single-step simulation. It emphasises using this customised run control to maintain the defined stochasticity models.

Right: The "Repertoire of Computation" panel, referred to as "Previously created Neuron morphology", appears to be a visualisation and simulation panel. It has sections for editing cell biophysics, gap junctions, synapses, and extracellular sources, as well as a range of simulation categories such as voltage, electric stimuli, and ion-specific dynamics. The panel also warns about integrating custom biophysics mechanisms into the simulation.

Manager of biophysical mechanisms.

The tool enables users to add and change various biophysical mechanisms to neurons to investigate their impact on neuron behaviour.

Upon clicking the "**Manage the distance of Mechanisms**" button located in the upper right corner (**Figure 10. Main Window**), the user shall be presented with two significant panels, as depicted in (**Figure 10**).

Instructions for the software panel Fig.14:

The software panel comprises two windows: the first (A) and the second (B).

Operating with Cell Compartments and Mechanisms in Window A:

In Window A, users can perform various operations on cell compartments and mechanisms to tailor their simulation:

1. Compartment Operations:

- Splitting and Merging Compartments:** Users can split one area into two or merge two areas into one. Additionally, renaming compartments is possible to enhance organisation.

2. Rescanning Model for Additional Mechanisms:

- Deep Rescan:** When adding extra areas to the cell (excluding from the BrainCell list), a deep rescan must include the changes effectively.
- Light Rescan:** A light rescan is essential for proper integration after adding extra mechanisms not listed in the BrainCell.

3. Export and Import Options:

- Export to JSON:** Users can create a JSON-format file containing biophysical mechanism data for future use or sharing.
- Import from JSON:** Previously saved mechanisms can be imported conveniently and efficiently.

These functionalities in Window A provide users with powerful tools to manage and enhance their simulation by modifying compartments, incorporating additional mechanisms, and maintaining a library of biophysical mechanisms for streamlined future use.

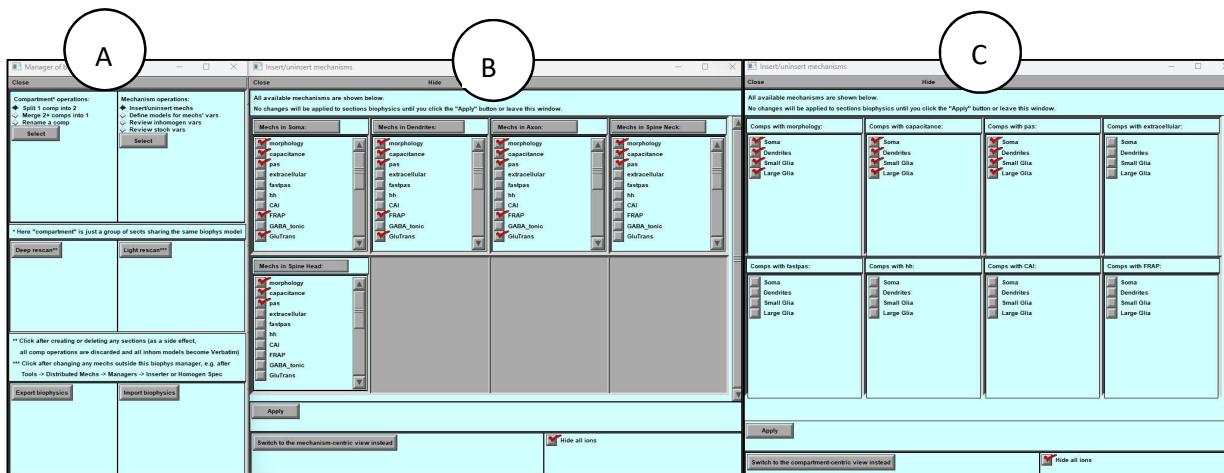


Figure 14. Panels for Biophysical Mechanisms Management

(A) Manager of Distributed Mechanisms Panel:

This panel facilitates efficient operation with compartments and mechanisms. Users can seamlessly work with various mechanisms in specific cell areas, add or divide different areas, and scan and enhance mechanism distribution. It also offers the flexibility to export/import different mechanisms, enhancing usability and customisation.

(B) Insert/Remove Mechanisms Panel:

This panel provides a comprehensive list of available mechanisms. Each cell area has its unique set of mechanisms.

(C) Insert/Remove Mechanisms Panel:

This panel is the same as B. However, each mechanism has its unique set of cell areas.

Users can swiftly utilise the "Apply" button to add selected mechanisms to the designated area. The down button allows users to switch between an area-centric and a mechanism-centric view, providing an adaptable interface for managing mechanisms effectively.

Window (B and C): Inserting and Removing Mechanisms and Cell-Part Operations

Window (B and C) is a pivotal tool that empowers users to manage and interact with mechanisms within specific regions of the brain cell (B) and specific mechanisms within different cell regions (C). Here's a breakdown of its features and functionalities:

1. Mechanisms Management:

- **Mechanisms Folder:** Contains a comprehensive list of available mechanisms for user selection.
- **Displayed Neuron Components:** Previously constructed neuron components are visually presented within this window.

2. Two Modes:

- **Initial Mode:** Displays mechanisms present in each neuron segment.
- **Secondary Mode:** Illustrates the precise location of each mechanism within the neuron segment.

3. Selecting Mechanisms:

- Users can effortlessly choose desired mechanisms by selecting corresponding check boxes, tailoring the simulation to their needs.

4. Cell-Part Operations:

- **Subgrouping, Merging, and Renaming:** Enables users to perform essential operations on different parts, enhancing cell structure organisation.

5. Interacting with Biophysical Mechanisms:

- **Mechanism Manipulation Panels:** Users can access panels allowing them to:

- Insert or remove mechanisms dynamically.
- Adjust spatial distribution of mechanisms for enhanced simulation control.
- Review mechanisms displaying spatial inhomogeneity, aiding in fine-tuning.
- **Stochastic Mechanism Analysis:** Facilitates analysis of stochastic mechanisms, providing insights into their behaviour within the simulation.

Window (B and C) is a comprehensive interface essential for managing mechanisms within specific brain cell regions and performing crucial cell-part operations. Users can seamlessly interact with, analyse, and modify mechanisms to achieve desired simulation outcomes.

Split, Merge and Remain option.

Splitting Cell Compartments

The **Split** function in BrainCell allows users to divide any part of a cell into multiple subparts, assign custom names to these subparts, and configure distinct biophysical mechanisms for each. This feature is particularly useful for specifying unique properties within different regions of a compartment, such as a dendrite.

Steps to Split a Compartment:

1. **Selecting the Compartment to Split**

- Choose a compartment to divide, for example, a dendrite.
- Click the **Split** button. A graphical representation of the selected compartment's geometry (e.g., the dendritic tree) will appear.

2. Selecting Subparts

- The software enables the selection of specific sections to include in the new subpart.
- Highlight the sections to be included in the new part. Selected regions will be displayed in red for clear identification, as shown in the figure.

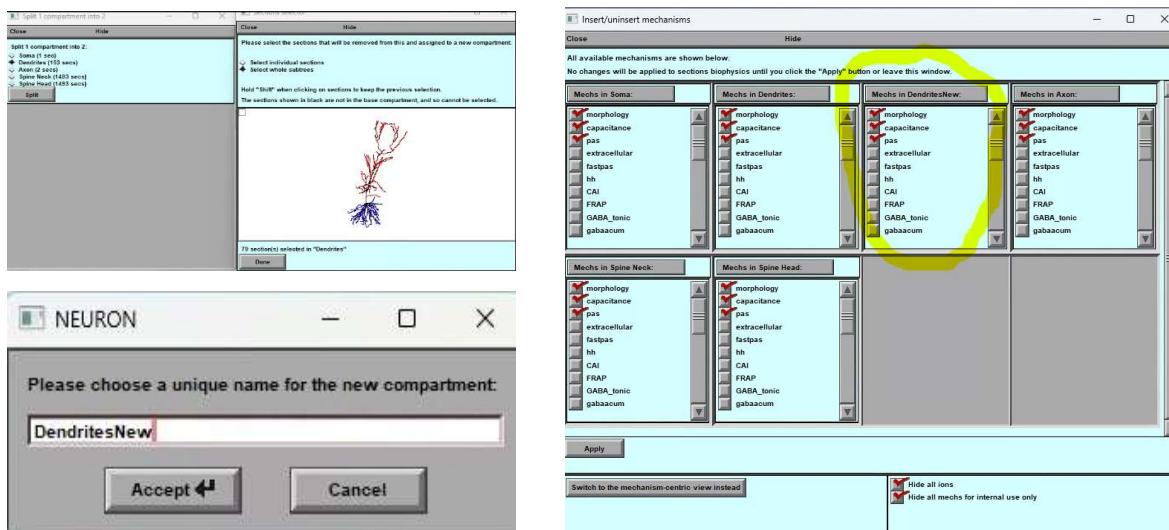
3. Naming the New Subpart

- After confirming the selection, click **Done**.
- A prompt will appear to assign a unique name to the new subpart (e.g., "DendritesNew"). Enter the desired name and press **Accept**.

4. Assigning Biophysical Mechanisms

- Once the subpart is created, it will automatically appear as a new compartment in the **Biophysical Mechanisms** panel.
- From here, you can modify or assign specific mechanisms (e.g., ion channels, capacitance, synaptic inputs) to the newly created subpart, distinct from the original compartment.

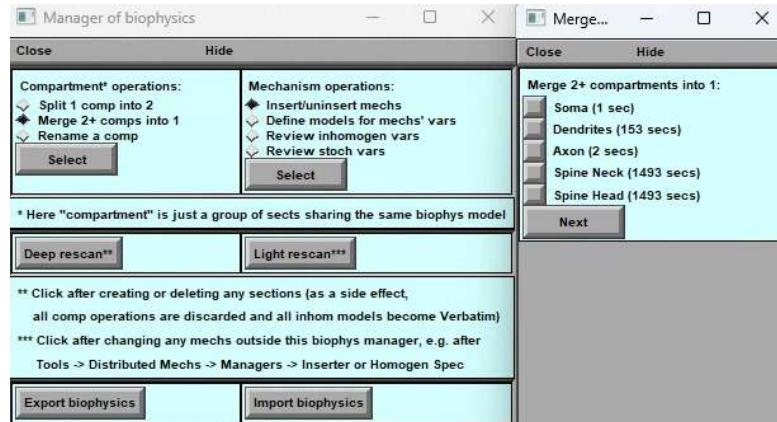
This functionality lets users model and simulate highly detailed and localised cellular behaviour. For example, you can divide a dendrite into 153 individual subparts and customise the physical properties of each, tailoring the simulation to match experimental data or explore hypothesis-driven scenarios.



Merging Cell Compartments

The **Merge** function in BrainCell allows users to combine two or more compartments into a single entity. This feature is ideal when simplifying a model or consolidating subcompartments to share a unified set of biophysical mechanisms.

Steps to Merge Compartments:



1. Accessing the Merge Function

- Open the **Manager of Biophysics** panel, as shown in the image.
- Under the "Compartment Operations" section, select **Merge 2+ compartments into 1**.

2. Selecting Compartments to Merge

- A list of existing compartments will appear (e.g., Soma, Dendrites, Axon, Spine Neck, Spine Head).
- Check the boxes next to the compartments you wish to merge. For instance, you could combine "Dendrites" and "Axon" into a single compartment.

3. Proceeding with the Merge

- Once the desired compartments are selected, click **Next**.
- The software will prompt confirmation of the operation. After confirming, the compartments will be merged into one, inheriting a single set of biophysical mechanisms.

4. Adjusting Biophysical Properties

- After merging, review the resulting compartment's properties under the "Mechanism Operations" section.
- You can modify or insert biophysical mechanisms (e.g., ion channels, passive properties).

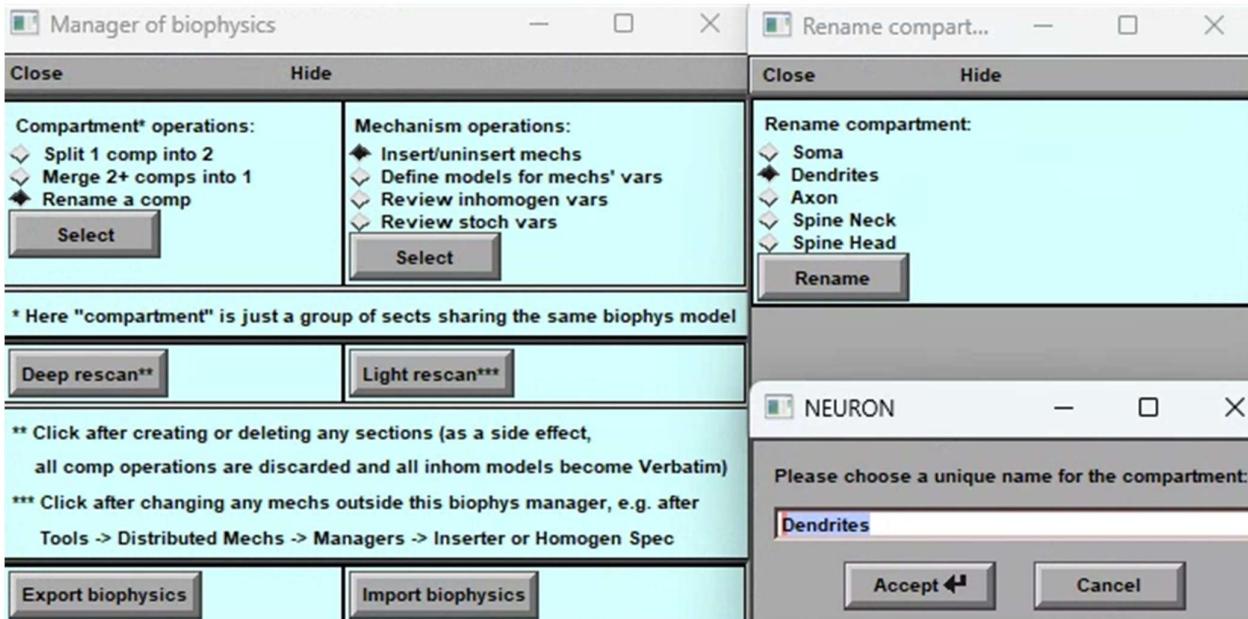
Notes on Merging:

- The **Deep Rescan** and **Light Rescan** buttons in the Manager of Biophysics help ensure that changes in geometry or mechanisms are applied accurately across the model.
- After merging compartments, verify the model integrity, as combining distinct sections may impact simulations.

This functionality is particularly useful for simplifying models where detailed compartmentalisation is not required or when consolidating regions for computational efficiency.

Rename Cell Compartments

The **Rename** function in BrainCell enables users to assign custom names to existing compartments, providing better organisation and clarity in models. This is particularly helpful when compartments represent specific functional or anatomical regions.



Steps to Rename a Compartment:

1. Accessing the Rename Option
 - o Open the **Manager of Biophysics** panel (as displayed in the image).
 - o Under the "Compartment Operations" section, click **Rename a comp**.
2. Selecting the Compartment to Rename
 - o A list of compartments will appear (e.g., Soma, Dendrites, Axon, Spine Neck, Spine Head).
 - o Choose the compartment you wish to rename by selecting it from the list.
3. Assigning a New Name
 - o A pop-up window will prompt you to enter a new, unique name for the compartment.
 - o Type the desired name (e.g., renaming "Dendrites" to "DendriticBranch1").
 - o Click **Accept** to save the new name.
4. Reviewing the Changes
 - o Once renamed, the updated compartment name will appear in all relevant model windows, ensuring consistency across simulations.

Notes:

- Ensure the new name is unique to avoid conflicts within the model.
- After renaming, it is recommended to perform a **Deep Rescan** or **Light Rescan** in the Manager of Biophysics to confirm that the changes are reflected throughout the model.
- Renaming does not alter the biophysical mechanisms or geometry of the compartment—it only updates the label for clarity.

This feature simplifies working with complex models by allowing more descriptive and intuitive compartment names.

Adjust the spatial distribution of mechanisms.

The central panel (**Figure 15**) adjusts the spatial distribution of the mechanism across any part of the cell, allowing the user different options to define the mathematical formula for the spatial distribution.

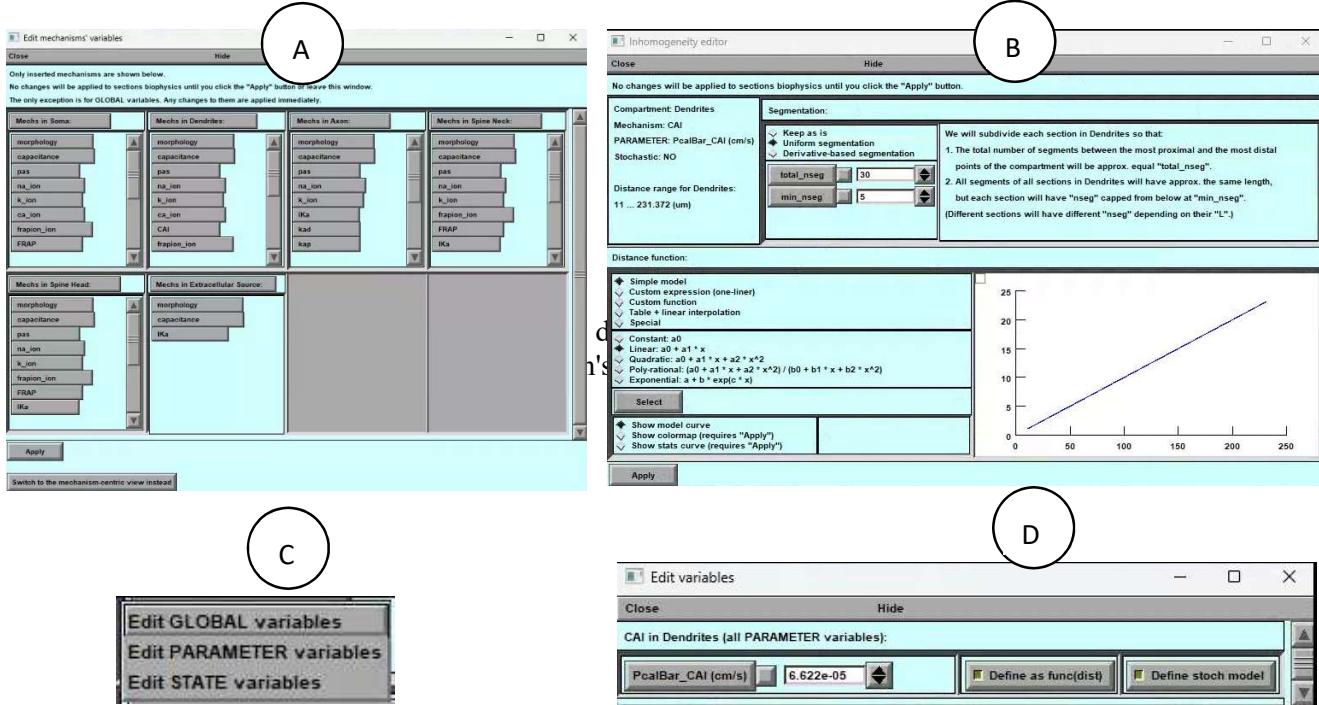


Figure 15: Panels for adding spatial properties to biophysical mechanisms in a cell. **A)** **Mechanism Selection Panel:** Users can select a biophysical mechanism and choose to modify its spatial. **B)** **Spatial Property Definition Panel:** Users can define the spatial properties of the selected parameters or variables. **C)** **Examples Panel:** Displays examples of the types of parameters and variables that can be modified for each biophysical mechanism. **D)** **Spatial Model Activation Panel:** Users can enable the spatial properties of the selected parameter or variable by pressing the "Define as a function" button.

The main interface (Fig.15A) is accurately designed to integrate with the various biophysical mechanisms in different neuron parts. Each segment of the neuron is conveniently located within its respective window in the main interface, containing all the biophysical mechanisms associated with it.

To change any mechanism, click on it, and a menu will pop up (Fig.15C), allowing you to select the desired editing options. This menu includes choices for global variables, state variables, and parameters. For more in-depth information about a selected variable, click on any of these options to access a separate window (Fig.15D).

In the new window, you'll see the current value of the variable if it's spatially homogeneous. If you want to make it spatially inhomogeneous, click the "**Define as a function of distance**" bar. Doing this will open a new window called the Heterogeneity Editor (Fig.15B).

In the **Heterogeneity Editor**, you can define the variable as a function of distance. This allows you to customise the properties of the variable based on the spatial location. Once you've described it, you can save your changes (Apply button) and edit other mechanisms.

Spatial Inhomogeneity of Biophysical Mechanisms Editor.

This editor (Fig.15B) allows us to work with neuron segments to determine the mechanisms' heterogeneity.

The editor's main window is divided into three parts. The upper part of the panel is where you can work with segments of neuron parts. This is a crucial part of the software because heterogeneity is determined not in each physical coordinate of the neuron but in each segment.

For example, if your dendrite has only one segment, no matter how long it is, it will be spatially non-uniform. Therefore, you must increase the number of segments for more detailed spatial heterogeneity. However, it should be noted that the more segments you have, the longer the calculation will take.

The spatial inhomogeneity specification feature of the software.

This feature allows you to specify the spatial inhomogeneity of a selected mechanism in your simulation. To use this feature, navigate to the central part of the window. Here, you will find the panel that offers five different modes for specifying the spatial inhomogeneity of your mechanism. Let's take a look at each of these modes:

Simple Model:

This mode offers several options for specifying your mechanism's spatial inhomogeneity. You can choose a constant value or specify a linear, quadratic, or polynomial function with two, three, or more parameters. You can also choose an exponential function.

Custom Function:

If you have a specific function in mind that is not covered by the options in the Simple Model mode, you can use the Custom Function mode. Here, you can enter any function you like, as long as it is written in either the neuron language or Python. To set your custom function, use the pop-up window.

Custom Function from File:

In addition to the Custom Function mode, you can specify a custom function from a file. This option allows you to define your function in a separate file, written in either Neuron or Python. Once you have created your file, you can specify it in the software and use it to specify the spatial inhomogeneity of your mechanism.

Table Function:

If you have experimental data that you would like to use to specify the spatial inhomogeneity of your mechanism, you can use the Table Function mode. This mode allows you to download your data either manually or as a text file and use it to specify the spatial inhomogeneity.

Special Function:

Finally, the "Special Function" option displays sections and the distribution of segments within the neuron cell. You have control over the cell model's discretisation stage. The discretisation is depicted on the cell's spatial graph. The segments are marked differently for better visualisation.

Visualisation of Spatial Heterogeneity

This part provides two different ways to visualise spatial heterogeneity. To access these options, navigate to the lower part of the main panel.

Option 1: Heterogeneity as the Distance from the Soma. With this option, you can visualise the degree of spatial heterogeneity related to the Distance from the Soma. This visualisation can help you identify areas of the neuron that exhibit high or low heterogeneity. To use this option, select it from the main panel.

Option 2: Spatial Colour map of Inhomogeneity on a cell. This option lets you visualise spatial heterogeneity using a colour gradient representing the cell's inhomogeneity degree. The colours range from cold to warm, with colder colours indicating lower levels of inhomogeneity and warmer colours indicating higher levels. To use this option, select it from the main panel.

Both of these visualisation options can provide valuable insights into your neuron's spatial heterogeneity. Choose the option that best suits your needs and explore your neuron's heterogeneity in new and insightful ways.

Adjust the stochastic distribution of mechanisms.

The central panel (**Figure 16**) adjusts the mechanism's stochastic properties across any part of the cell, allowing the user to define the mathematical formula for the stochastic distribution using different options.

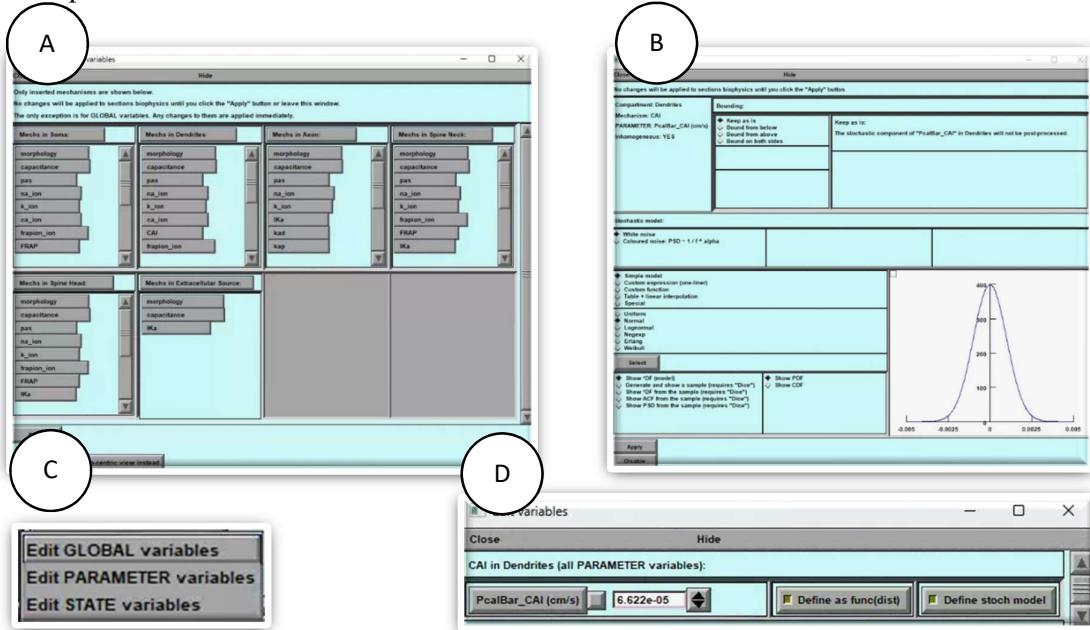


Figure 16: Panels for adding stochastic properties to biophysical mechanisms in a cell.

A) Mechanism Selection Panel: Users can select a biophysical mechanism and choose to modify its stochastic properties.

B) Stochastic Property Definition Panel: Users can define the stochastic properties of the selected mechanism's parameters or variables.

C) Examples Panel: This panel displays examples of the types of parameters and variables that can be modified for each biophysical mechanism.

D) Stochastic Model Activation Panel: Users can enable the stochastic properties of the selected parameter or variable by pressing the "Define stochastic model" button.

To begin editing any mechanism, click on it. This will bring up a menu where you can select what you want to edit. The menu includes options for global variables, state variables, and parameters. You can access another window that provides more detailed information about the selected variable by clicking on any of these options.

In the new window, you'll see the current value of the variable if it's spatially homogeneous and "nan" is spatial non-uniform. To make this mechanism stochastic, click the "Define as a stochastic model" bar. Doing this will open a new window called the **Stochasticity Editor**.

Stochasticity Editor

You can define the variable as a different stochastic function in the Stochasticity Editor. This allows you to customise the behaviour of the variable as a stochastic function in time. Once you've described it, you can save your changes and edit other mechanisms.

The **Stochasticity Editor** window has four parts to help you model and visualise different stochastic distributions for a given variable. Let's go through each part in detail.

1) Upper Part:

a) *Left part*: This part shows information about the state, “**Inhomogeneous**” of stochasticity of this variable/parameter, “**Mechanisms**”, its location on the cell,” Compartment”, and full name with units, “**PARAMETER**”. It also indicates whether this variable/parameter is spatially inhomogeneous.

b) *Right part “Bounding”*: Here you can select the parametric domain of (**DF**) density function of the stochastic variable, either non-infinite “**Keep as is**”, from above “**Bound from above**”, from below “**Bound from below**”, or from both sides, “**Bound from both sides**”. The full definition of any mode is on the right of the panel.

2) **Stochastic Model**: This part allows you to select the type of stochasticity, either “**White noise**” or “**Coloured noise**”, with PSD of different alpha correlation.

a) *Simple Model*: This part includes standard models such as “**Uniform**”, “**Normal**”, “**Logarithmic**”, “**Exponential**”, “**Erlang**”, and “**Weibull**” distributionы. You can select any of these models. The selection of any distribution is accompanied by a pop-up panel containing the distribution's corresponding parameters.

b) *Custom expression*: You can add various stochastic functions by writing in line. **(For premium users)**

c) *Custom expression*: You can add your various stochastic functions by writing them down and including them in "hoc" files. **(For premium users)**

d) *Table and linear interpolation*: You can add the experimental data to a table. **(For premium users)**

e) *Special Functions*: The user is given a unique set of simple functions to test stochastic variables. The function **(1,0,0,...)** is a numerical delta function with a uniform spectrum distribution. The function **(1,1,1, ...)** is a function with zero frequency. The function **(1,0,-1,1,0,-1, ...)** is an essential periodic function, cosine with half-Nyquist frequency. The function **(1,-1,1,-1, ...)** is a basic periodic function cosine with Nyquist frequency. Function **Foo** is an essential periodic function.

3) **Visualize and Model Part**: This part allows you to model and visualise the selected type of stochastic distribution. You can:

a) Visualize the distribution density (**DF**) function for infinite trials.

b) Generate a sample of random numbers according to the chosen function.

Several are indicated on the panel.

c) Visualize the density function (**DF**) for this sample and the ideal function.

d) Build the autocorrelation function (**ACF**) of this sample.

e) Build this sample's power spectral density (**PSD**) function.

4) **Apply or Disable Part**: In the end, two buttons allow you to accept or refuse the stochasticity of this variable.

Important. The stochastic dynamics can be computed using a special run control panel, “**AltRunControl**”, provided by “**BrainCell**”.

Manager of synapses.

The following information is vital for those who wish to create synaptic inputs.

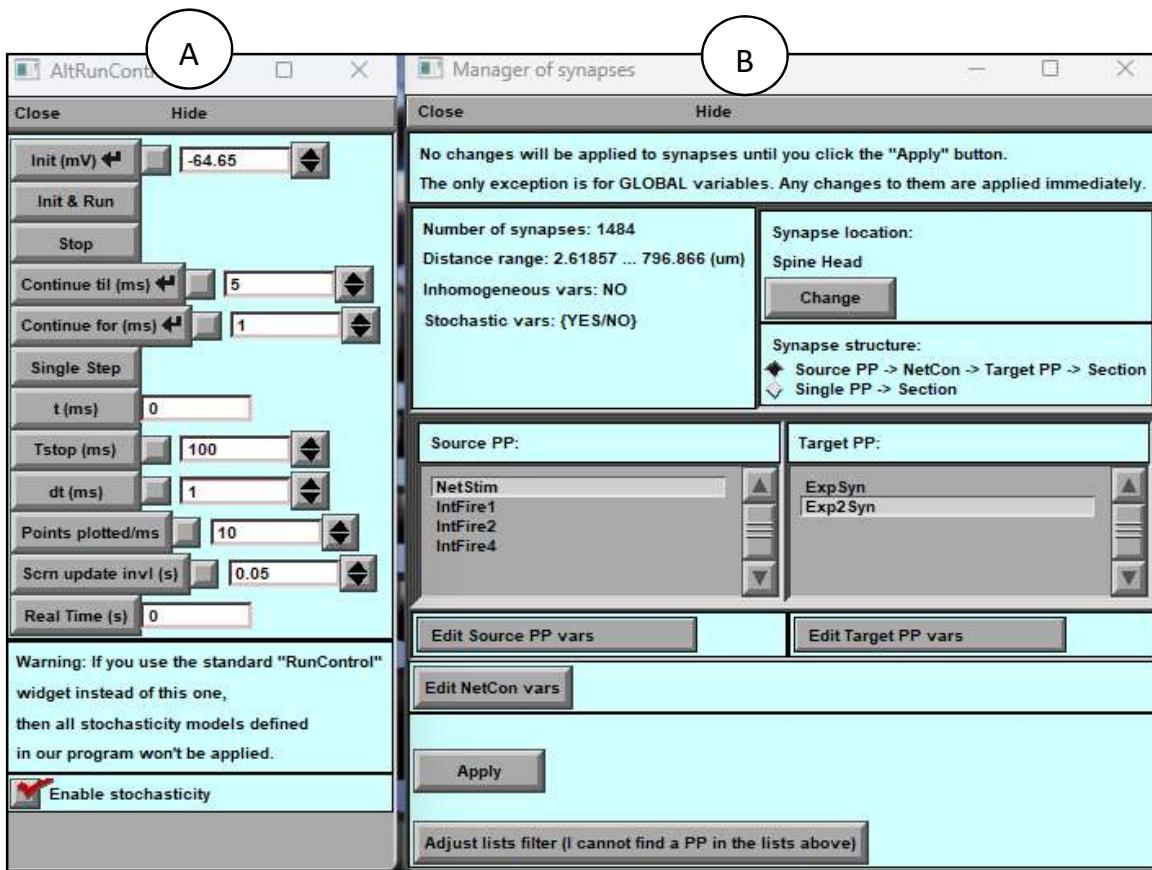


Figure 17. The Synaptic Simulations. A) Panel of Alternative Run Simulation for Stochastic Variables: The panel demonstrate alternative run simulations for stochastic variables within the synaptic environment. **B) Panel of Manager of Synaptic and Presynaptic Mechanisms:** The manager acts as a central control system, orchestrating the interactions between different synaptic elements and ensuring efficient communication within the neural network (Details in the text).

The synaptic panel (Fig.17B) produces and distributes distinct synaptic inputs throughout a dendritic tree. The number of spines on the dendritic tree determines the number of synapses without excess or deficiency. To modify the distribution and density of spines, one must use the main panel (Fig.10). Please note that the number of spines (synapses) on the synaptic panel (Fig.17B) cannot be altered. However, with this panel, users can relocate synapses, either on the spine itself or near it on the dendrite. The distribution of synapses may vary in proportions, with some distributed over the spines and others located close to them. When a synapse is near a spine, the spine's conductance becomes zero, essentially disappearing in an electrical sense. However, if the synapse is situated on a spine, it functions as a full-fledged conductor. When a spine is positioned next to the synapse, it can affect synaptic function.

Note. To compute the synaptic release probability, use the **AltRunControl** and indicate the key “**Enable stochasticity**.”

This **Synaptic panel** (Fig.17B) is designed to give users a comprehensive understanding of the synapse's role. To get started, look at the upper portion of the panel. You'll find important

information and tools to manipulate the location of synapses. The **Synaptic panel** has three parts to help you model and visualise different synaptic distributions and efficacy types.

Let's go through each part in detail.

1. [Synaptic manager upper part.](#)
2. [Synaptic manager middle part.](#)
3. [Synaptic manager bottom part.](#)

Synaptic manager “upper part”.

The information section in the upper portion of the panel is where you can find valuable data about the synapses on the dendritic tree. This section will indicate the number of synapses distributed throughout the dendritic tree, including the spatial range and whether the parameters are spatially homogeneous. Additionally, it will provide you with stochastic variables of its parameters, which is crucial for understanding the behaviour of the synapses.

To change the location of the synapses on spines or dendrites, use the tools located in the upper right portion of the panel. By manipulating these tools, you can adjust the position of the synapses to your desired location.

The “Synapse location” button opens a pop-up panel (**Fig.18A**).

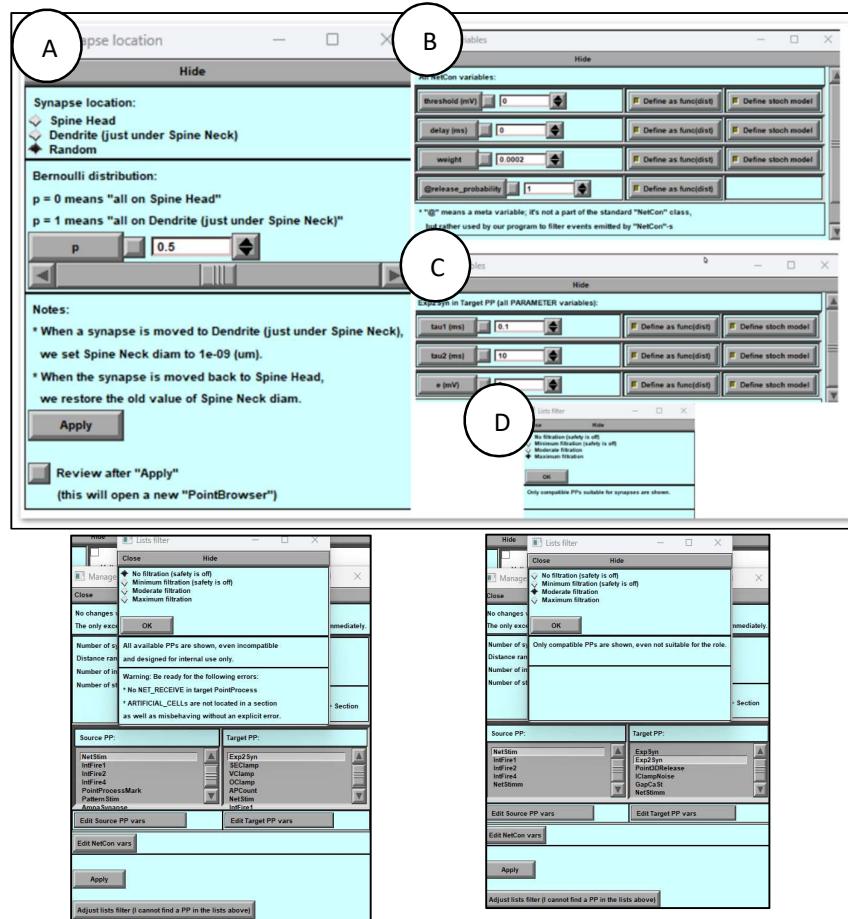


Figure 18: Pop-Up Panels of Synaptic Manager. A) shows the synaptic location manager, allowing users to visualise the spatial distribution of synapses. B) displays the manager of presynaptic signals, which provides information about the presynaptic neuron's activity. C) presents the manager of synaptic parameters, where users can modify synaptic properties such as strength and plasticity. D) shows the manager of presynaptic and postsynaptic mechanisms, located in the mechanism directory, which enables users to customise the synaptic transmission and reception mechanisms.

This part guides you through this panel's upper and lower (**Fig.18A**) parts and helps you understand their functionalities.

Upper Part: The upper part of the “**Synapse location**” panel enables you to modify the location of the synapses. There are three options available on this panel that you can use to change the location of synapses:

1. Place all synapses on the spines.
2. Place synapses on dendrites at the location of spines
3. Place synapses on dendrites and spines in different proportions

You can select any of the above options according to your requirements, and the software will make the necessary changes accordingly.

Lower Part: The lower part of the Synapse panel contains informational data and two buttons: "**Apply**" and "**Review after Apply**".

The informational data in the lower part provides details about the synapses' current location and density. It also shows the changes made to the synapse location after selecting any options mentioned in the upper part of the panel.

The "**Apply**" button confirms the changes made to the synapse location and saves them in the software. If you do not wish to save the changes, click the "**Cancel**" button.

The "**Review after Apply**" button shows how the program changed the synapse location. This option allows you to verify the changes and ensure they align with your requirements.

Synaptic structure.

*Synaptic manager “top part” (**Figure 18 B**).*

This part creates synaptic structures in the neuronal model (**Figure 18**) **Synaptic panel (B)**. Synapses are two types in "BrainCell", one that requires a presynaptic signal and the other that works without a presynaptic signal. The second type turns on depending on the "**onset**" parameter.

The panel (**Fig. 18**) offers two synaptic structures to accommodate these two types. The first structure includes presynaptic and postsynaptic mechanisms (**Source PP → NetCon → Target PP → Section**), while the second includes only postsynaptic mechanisms (**Source PP → Section**). Selecting one of these scenarios allows you to get either postsynaptic or presynaptic and postsynaptic mechanisms in the middle panel.

Please note that you cannot use the "probability of release" parameter if you choose only the presynaptic option (**Source PP → NetCon → Target PP → Section**).

Synaptic manager “middle part”

This middle section of the panel (Fig. 18 B) has been specifically designed to allow you to organise and manipulate the presynaptic and postsynaptic functions of synapses with various parameters. Here, you can choose different presynaptic mechanisms (left panel – Source PP) and connect them to the synaptic mechanism (right panel – Target PP).

Mechanism Panels:

The user interface displays two panels: the left panel showcases mechanisms associated with presynaptic function, and the right panel presents mechanisms associated with synaptic function. These panels provide access to NEURON mechanisms and unique mechanisms in the BrainCell directory.

Finding Unique Mechanisms:

*If you cannot locate a specific unique mechanism in the panel, you can utilise the "**Adjust lists filter**" feature. A new popup window will appear by pressing this unique key, allowing you to apply specific filters. The filtering functionality lets you refine your search and view all possible point mechanisms within the BrainCell mechanisms folder. In (**Figure 18**), two panels are depicted,*

each showcasing a different level of filtering. This feature lets you quickly identify and insert unique mechanisms into your neuronal model based on your requirements.

Once you have selected the appropriate presynaptic and postsynaptic mechanisms, you can start modifying the set of parameters by clicking on the buttons labelled “**Edit source PP vars**” and “**Edit target PP vars**”.

The modification of the spatial distribution of Point base (**Synaptic**) mechanisms follows the same rules as the modification of the spatial distribution of standard density mechanisms, as explained in the section. [Manage distribution mechanisms](#).

The randomness of synaptic parameters changes specifically when the presynaptic signal is generated, as opposed to the randomness of spatially distributed mechanisms. This means the synapse's stochastic events only occur when the presynaptic signal is generated or when the "onset" time parameter value is reached. However, the panel for controlling stochasticity is no different from that for spatial mechanisms.

User Manual Note:

It's crucial to highlight that within this mode (**Source PP → Section**), users can incorporate various point mechanisms. These may include adding an electrode in the potential fixation mode or applying an external current—virtually any modification defined as a localised alteration in current or potential can be introduced in this context.

.....

Synaptic manager “bottom part”.

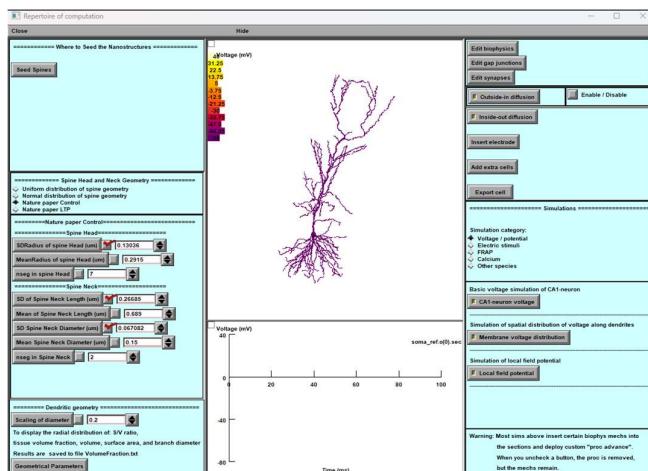
To manipulate the "[NetCon](#)" mechanism, which connects the presynaptic and synapse functions via four parameters - "**threshold**", "**delay**", "**weight**", and "**release probability**" - we have designed a specific section at the bottom of the panel, featuring a button labelled "**Edit NetCon vars**" (Fig.B). This section ensures seamless coordination between the presynaptic and postsynaptic functions.

In addition, you can use the "**Adjust list filter**" button (Fig. 18D) to select and add various synaptic mechanisms that are not yet included in the Middle panel. This feature allows you to add your biophysical mechanism and use it to form new synaptic connections, unlike the standard synaptic mechanisms of “**Neuron**”. New mechanisms should be uploaded to the "Mechanisms" section/directory.

To accept all modifications with synaptic connections, click the "**Apply**" button in the same bottom section.

Spatially - non-uniform sources of extracellular ions.

This section provides detailed instructions for organising the calculation of ion concentration near a model cell in BrainCell, regardless of the concentration source.



Main Panel Functions:

The main panel features two key buttons: **Outside-In Diffusion** and **Inside-Out Diffusion**.

1. Outside-In Diffusion:

- This option enables users to create and manage different ion concentration sources outside the cell.
- Steps to use:
 1. Click on "Outside-In Diffusion".
 2. Define the type and properties of the ion concentration source.

3. Configure spatial and temporal parameters to simulate diffusion towards the cell.

2. Inside-Out Diffusion:

- This option calculates the ion concentration dynamics that the cell releases.
- Steps to use:
 1. Click on "Inside-Out Diffusion".
 2. Specify the type of ion and its release dynamics.
 3. Set up the simulation to observe how the released ions affect the surrounding environment over time.

These features allow for comprehensive modelling and simulation of ion concentration changes around a model cell, providing insights into both external and cell-released ion dynamics.

The "Neuron" software starts with a uniform distribution of extracellular ions. Still, sometimes researchers need to study the specific spatiotemporal dynamics of these ions, including different types, neurotransmitters, and neuromodulators. To help with this, BRAINCELL has added

a feature **Edit extracellular sources (in development)** that lets users create different scenarios for ion dynamics. To access this feature, look for the option in the upper right corner of the main panel and select it. This will take you to the extracellular ion distribution editor. It's important to remember that using this editor doesn't consider the potential effect of neuron feedback on ion concentrations.

However, it allows researchers to conduct more detailed investigations into ion dynamics, leading to a better understanding of how neurons behave and interact in the extracellular environment.

Outside-In Diffusion

The Inside-Out Diffusion (Figure 33) is a powerful tool for simulating extracellular ion dynamics release by **external courses**. This chapter will guide you through its key features and how to use them effectively.

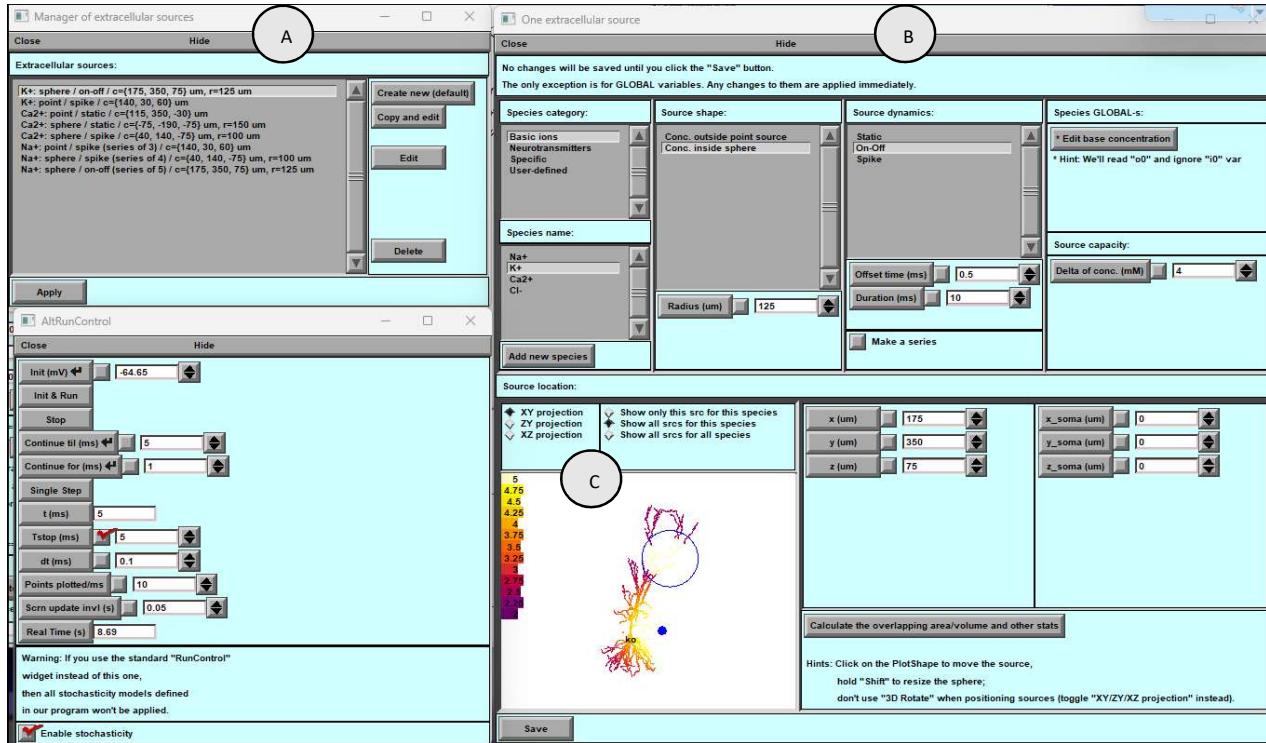


Fig. 19. External ionic concentration

A) The existing external sources panel is a convenient tool for viewing and managing previously created external sources. Users can select an external source from this list to edit or delete it.

B) The external source creation panel allows users to design software using three templates: point single release, point constant diffusion, and spherical area of concentration. Users can specify the location and properties of the external source.

C) The visual graphical panel allows users to specify the coordinates and area of an external source by dragging and dropping. Users can also use Shift to specify the radius of the external source.

Overview (Figure 19): Users can precisely and flexibly manipulate the extracellular ion concentrations, accommodating various spatiotemporal requirements within the system.

1. Modifying Ion Concentrations:

- Users can simultaneously create distinct sources of external ion concentrations, allowing for potential spatial and temporal overlap.
- The flexibility extends to generating varied sources of identical ion concentrations, separated or overlapping in space and time.

2. Using the Functionality:

- After making changes, users must remember to save them by pressing the "Save" button and clicking "Apply" on Panel A to implement the modifications.

Panel A - Extracellular Concentration Changes:

- The central panel displays all alterations in extracellular ion concentrations.
- Enables addition, editing, or deletion of sources of concentration changes.

Panel B - Ion and Concentration Changes:

- Allows users to specify a type of ion and modify its concentration.
- Built-in options include major ions (sodium, potassium, chlorine, calcium), neurotransmitters (acetylcholine, glutamate, GABA), and specific ions (FRAP and inositol triphosphate).
- Users can define their custom ions (Ion 1 or 2).

Source Shape Panel:

- Users can define the spatial distribution of ions within or outside a spherical area.

Source Dynamics:

- Users can set three types of ion dynamics over time: constant activation, instantaneous activation, or toggling on and off.
- Each source has adjustable parameters accessible in the settings window.

Creating Concentration Series:

- Users can create a series based on the concentration change time by enabling the "Create series" option.
- Customise series parameters such as interval start number and add noise for realistic interval variations.

Spatial Localisation:

- Allows users to position the concentration source spatially.
- The panel offers visualisation (in colour) in various planes (XY, XZ, YZ) and different ion views (individual ion, type of ion, or all ions).
- Graphical representation displays extracellular concentration dynamics upon user interaction (use the right mouse key to choose "**Extracellular Concentration dynamics graph**").

Users can comprehensively study and manipulate ion dynamics by utilising these features, enhancing their understanding of neuron behaviour and interactions within the extracellular environment.

For further details and interactions, refer to the graphics window's graphical interface and right-click options for extracellular concentration dynamic graphs.

Basic set of equation for single on-off release :

For a single point source with “on-off” switch dynamics, the extracellular concentration (mM) is given by the equation:

$$O_{src}(t, Distance)$$

$$= O_{spc\ basic} + \frac{ss_{src} Radius_{src}}{Distance} (timeFactor(t - t_{start}) - timeFactor(t - t_{end}))$$

Where:

t – the observation time (ms);

Distance – the distance between the source point and the observation point (μm);

O_{spc basic} – the basic extracellular concentration that takes place when there is no source (mM); this is the species parameter (Na⁺, K⁺ etc.) rather than the source parameter;

ss_{src} and Radius_{src} – specific surface concentration (mM) and specific radius (μm) of the point source respectively;

tstart and tend – the time when the source turns into the active and then inactive state respectively (ms); tstart < tend;

timeFactor(Δt) – an auxiliary function (unitless) given by:

$$\text{timeFactor}(\Delta t) = \begin{cases} \text{erfc}\left(\frac{\text{Distance}}{2\sqrt{\text{Diff}_{spc} \Delta t}}\right) & \text{if } \Delta t \geq 0 \\ 0 & \text{if } \Delta t \leq 0 \end{cases}$$

Diff_{spc} – the diffusion coefficient given species and given temperature (μm² / ms);

erfc() – the complementary error function; erfc(0) = 1; erfc(∞) = 0.*

Manager of Extracellular Sources Panel

Description: When the user activates the extracellular sources function, the first panel displays all the current ion concentration sources in the neuron cell. Initially, several predefined sources are available but remain inactive until manually activated by the user.

Instructions:

1. **Viewing Sources:** The panel shows a list of all available ion sources.
2. **Activating Sources:** Activate each source to start ion release.
3. **Editing Sources:** Double-click any source to open the main configuration panel (Panel B).
 - **Panel B Features:**
 - Change ion types
 - Modify spatial distribution
 - Adjust time dynamics
 - Set basic parameters and properties

Use these tools to customize the extracellular ion environment around your model cell effectively.

Using the Single Ion Panel

This panel allows you to select predefined ions or create custom ions to simulate their behavior within a cell model.

Predefined Ions

The BrainCell includes a library of common ions, categorized as:

- Main Ions: Potassium (K+), Sodium (Na+), Chlorine (Cl-), Calcium (Ca2+)
- Neurotransmitters: Acetylcholine (Ach), Gamma-aminobutyric acid (GABA), Glutamate (Glu)
- Helper Ions: Inositol trisphosphate (IP3), Fluorescence recovery after photobleaching (FRAP)
- Creating Custom Ions

Use the built-in panel to define ion properties and spatial-temporal dynamics:

Spatial Distribution:

Determines how ions are released outside the cell:

- Point Source: Releases ions from a single point outward.
- Spherical Source: Releases ions uniformly within a defined sphere.
- Additional Distributions: Configure more complex release patterns as needed.

Temporal Dynamics: Controls ion concentration changes over time:

- Static: Maintains a constant concentration.
- On/Off: Turns the ion source on/off at specific times.
- Instantaneous: Releases a defined amount of ions at a specific time.
- Configure parameters like release radius and start time for concentration changes.

Global parameters Settings

Diffusion Coefficients: Define how quickly ions move outside the cell.

Temperature Dependence: Simulate how temperature affects diffusion coefficient.

Ion Absorption: Enable a model for ion uptake by extracellular space using a linear function.

Defining Spatial Distribution

Graphical Interface: Use the mouse to directly manipulate the position of your ion sources within the visual representation of the cell. Drag and drop them to their desired locations within different cell planes.

Numerical Values: Prefer a more precise approach? Enter specific coordinates (X, Y, Z) for each ion source to achieve exact placement.

Move the Soma: Shift the entire soma at specific coordinates (X, Y, Z).

Saving Ion Configuration

Once you have defined your custom ion properties, click the "Save" button to add it to the main ion selection panel for easy access in your simulations.

Don't forget to "save" and "apply" your changes.

Configuring Inside-Out Diffusion Simulations

The Inside-Out Diffusion (Figure 33) is a powerful tool for simulating extracellular ion dynamics released by cells. This chapter will guide you through its key features and how to use them effectively.

1. General Settings (Panel A):

Begin by selecting your simulation type and computational resource. Configure the extracellular space parameters carefully, as these significantly impact your results. The nano-geometry toggle and precision settings allow to create an cation's accuracy and computational demands.

2. Diffusion Management (Panel B):

Choose between on-the-fly or deferred calculations based on your simulation needs. Select and configure the ion species relevant to your study. The spatiotemporal grid and recording period settings are crucial for balancing detail and computational efficiency.

3. Simulation Control (Panel C):

Use the AltRunControl Panel to manage runtime parameters, set initial conditions, and control simulation progression. This panel also allows you to introduce stochasticity, adding realism to your simulations.

4. Visualization Configuration (Panel D):

This panel is key for analysing your results. Choose your preferred projection and grid type. Use the interactive display to precisely define your region of interest. Adjust resolution for optimal visualization of ion concentrations.

Pro Tip: When defining your grid region, use the XY/YZ/XZ projection options for accurate placement, rather than 3D rotation. By mastering these panels, you'll be able to conduct sophisticated extracellular diffusion simulations, providing valuable insights into ion dynamics in cellular environments.

Remember to refer to the "Read Me" buttons for detailed instructions on each feature.

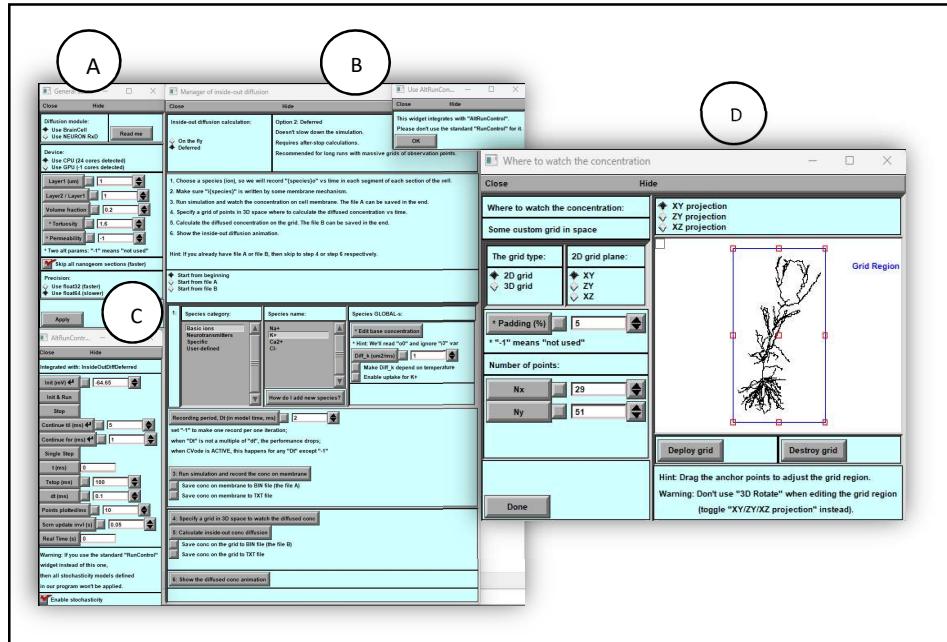


Figure 20: Inside-Out Diffusion Control Panel for Extracellular Simulation

A. General Settings

- Select simulation type: NEURON RxD or BrainCell
- Computation resource: CPU or GPU
- Extracellular space configuration:
 - o Layer dimensions (pre membrane and exchange layers)
 - o Volume fraction
 - o Tortuosity
 - o Permeability (optional parameter)
- Nano geometry toggle
- Precision setting (64 or 32-bit)

B. Inside-Out Diffusion Manager

- Calculation mode: On-the-fly or Deferred
- Computation initiation point
- Ion species selection and configuration:
 - Main ions (K⁺, Na⁺, Cl⁻, Ca²⁺)
 - Neurotransmitters (ACh, GABA, Glutamate)
 - Helper ions (IP₃, FRAP)
 - Custom ion creation option
- Spatiotemporal grid definition
- Recording period specification
- Simulation execution and data storage options
- Concentration visualization settings (See D)
- Fixed-concentration extracellular space calculation option
- Movie generation settings for concentration visualization

C. AltRunControl Panel

- Simulation runtime parameter control
- Initial condition setup
- Simulation progression management
- Recording period and data saving options released by cells enablement and management

D. Concentration Visualization Configuration Panel

This panel allows users to customize the visualization of ion concentration data in the simulation.

- Projection selection: Choose between XY, XZ, or YZ projections for 2D visualization.
- Grid type: Select between 2D plane or 3D grid for concentration mapping.
- Axis selection: Enable or disable X, Y, and Z axes for the visualisation.
- Padding adjustment: Fine-tune the padding percentage around the visualised area.
- Grid region definition: Interactive graphical interface to set the region of interest.
- Resolution control: Adjust the number of points along the Nx and Ny axes for visualisation granularity.

The central display shows a neuron model with an adjustable grid overlay, allowing users to define the region for concentration analysis precisely. **Note:** Use the anchor points to adjust the grid region. When editing the grid region, use "XY/YZ/XZ projection" instead of "3D Rotate" for accurate placement.

Note: Refer to the "Read Me" information button in each section for detailed operation instructions.
These panels collectively enable detailed configuration and execution of inside-out diffusion simulations, allowing users to model ion release and behaviour in a cellular environment effectively.

Gap Junctions in BrainCell.

Gap Junction Sets in Astrocytes

Brain Cell software allows users to configure and manage Gap Junction sets in Astrocytes, offering customization in biophysical properties, spatial distribution, quantity, and stochastic characteristics. Users can independently edit each Gap Junction set to fine-tune their simulations.

Upon accessing the Gap Junction feature, two panels will be presented:

- The primary panel lets users add or remove Gap Junction sets.
- An auxiliary panel serves to facilitate navigation between this management panel and the calculations panel, which is optimized for accurately computing stochastic processes.

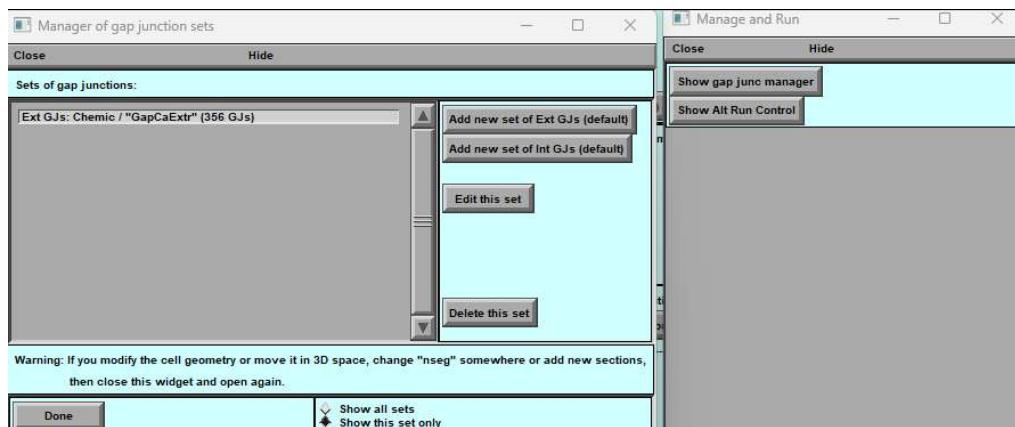


Figure 21. Left Panel - Manager of Gap Junction Sets:

A list of existing Gap Junction sets, each potentially with a unique identifier, like "Ext GJs: Chemic / "GapCaExt" indicating a set of 356 Gap Junctions.

Options to add a new set of external or internal Gap Junctions (GJs) with default settings.

Functionality to edit or delete the selected set of Gap Junctions.

A reminder that if the cell geometry is modified, "nseg" must be changed or new sections added, followed by reopening the widget for updates to take effect.

Buttons are to show either all sets or only the selected set.

A "Done" button to exit the manager.

Right Panel - Manage and Run: Options to show the gap junction manager or the run control panel for simulations.

Parameters of gap junctions in Neurons and Astrocytes.

This user manual section will provide detailed instructions on creating different types of Gap Junctions (GJ) within cells using BrainCell software. These GJ connections can be established in both astrocytes and neurons. It's important to note that there is a critical distinction between these two cell types: in astrocytes, you can create internal GJ connections within the same cell and external GJ connections directed to other cells. In contrast, in neurons, you can only create external GJ connections.

Let's begin by exploring the main window, which is depicted below.

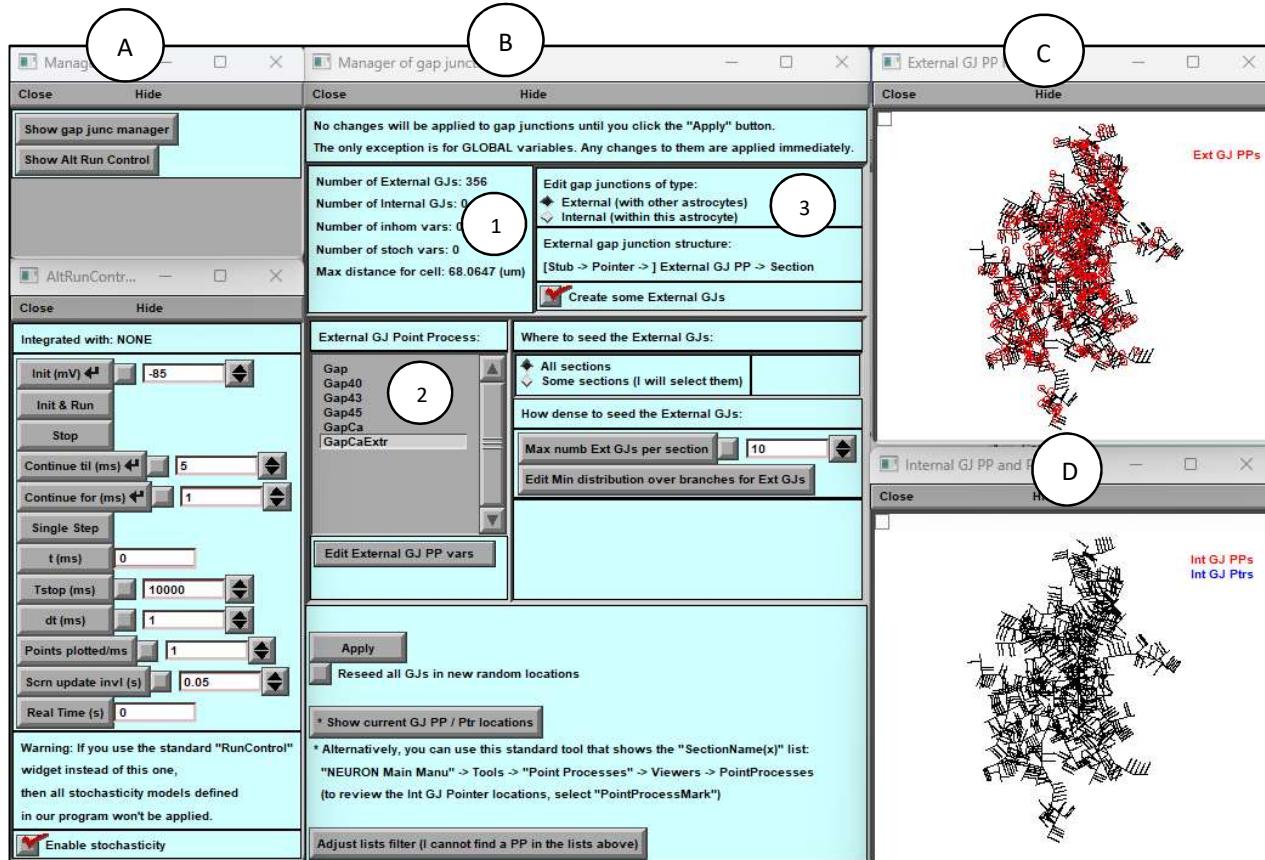


Figure 22. Main Window for Gap Junction (GJ) Creation and Editing

A) **Two Support Windows.** The small support window allows you to access the main window and the calculation management window even if they are accidentally closed.

B) Main Window:

- 1) Provides information about the number and types of GJ, along with brief reminder instructions.
- 2) Allows you to select GJ types described in your mod files. The displayed files are limited by certain filters. If your files are not visible, utilize the "Adjust List Filter" below to expand the list. Additionally, there is a "Edit external GJ" button that enables you to modify the selected GJ parameters.

3) This set of panels lets you select the type of GJ (internal or external) and distribute them across various parts of the cell with varying densities. You can specify the maximum number and density of these GJ, which can be either spatially uniform or spatially varying.

C) Auxiliary Panels. These panels visually depict the distribution of different GJ types within the cell and provide details on their arrangements.

Adding and Modifying Gap Junctions in BrainCell

BrainCell software allows you to add and adjust Gap Junctions (GJs) to customize your cell models. Here's a step-by-step guide:

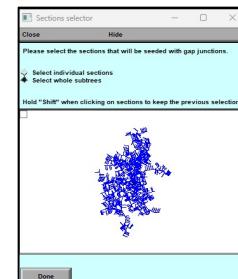
Initial Setup:

1. A basic set of external GJs is automatically inserted when you start the program. These GJs transfer calcium linearly from external cells to a selected astrocyte. A dedicated MOD file is used for this purpose.
2. The MOD file is designed so that when potassium is in equilibrium inside the cell under normal conditions, there is no calcium flow between this astrocyte and the external cells. The distribution of these GJs is random and follows a typical distribution pattern with specified density and a maximum number, as visible in panel 3.

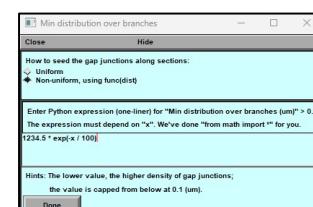
What You Can Do:

1. **Replace MOD File:** You can replace the original MOD file with your own while keeping the original GJ distribution and density intact.
2. **Change GJ Design and Spatial Distribution:**

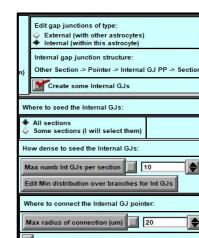
- Navigate to panel 3. Here, you can utilize the distribution function (e.g., for External GJs) and choose between "All section" or "Some section."
- The "Some section" option opens graphical windows where you can select the locations for GJ distribution by clicking on the cell sections. Hold the Alt-key to select multiple sections.



- Adjust the type of density and distribution for GJs. The maximum number is initially set to 10 per section, but you can change it to 0 or Infinity. If you set it to 0, no GJs of this type will be in your cell.
- You can modify the density of GJs (initially set at 8.4376) within a range of 0.1 to Infinity or choose non-uniform density using the "Edit Min distribution of the branches" function.
- For non-uniform spatial distribution of GJs, use the "Non-Uniform" function, which opens a new panel. Here, you can define a formula based on the x distance to distribute the GJs. Click "Done" to see the new distribution.

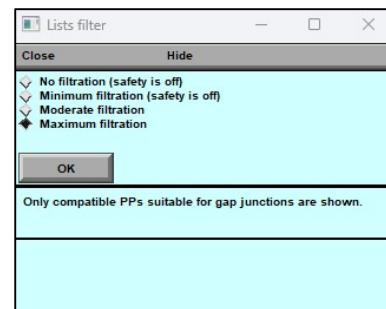


3. **Create Internal GJs:** Panel 3 functions differently when creating internal GJs. You can specify the minimum distance between cell parts where internal GJs may connect.



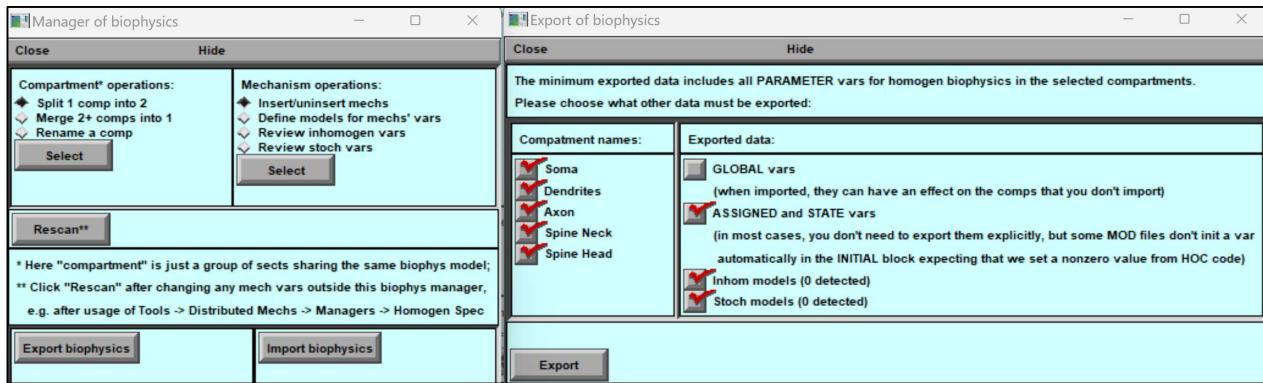
4. **Filter MOD Files:** In the MOD-file directory, you'll find various mod files that can be interpreted as GJs. However, not all of them can be used as gap junctions. To assist you, we've introduced a filter that helps you select the appropriate mod file. The default filtration is well-suited for GJs, but you can adjust it if you wish to use a particular mod file.
5. **After** making your desired adjustments, click the "Apply" button, and your cell will have a set of Gap Junctions according to your specifications.

Customizing Gap Junctions in BrainCell allows you to create detailed and biologically relevant cell models tailored to your research needs.



Exporting and Importing Biophysical Mechanisms with BrainCell Software

Our BrainCell software offers a robust feature enabling users to efficiently export and import biophysical mechanisms individually. This unique characteristic simplifies the process of saving sets of biophysical mechanisms for a cell and utilising different cell shapes for different studies, such as analysing the impact of neuron shapes on output discharge. The export and import functionality are conveniently located within the "biophysics manager" panel.



Export Functionality

When exporting, the user can selectively choose and export mechanisms associated with specific cells. This information is then stored in a designated text file. The file's structure is outlined below, providing clear insights into the placement of each mechanism and its respective parameter values.

Export Text File Structure

```
},  
    "pas": {  
        "PARAMETER": {  
            "g_pas": 8.9686098654  
        },  
        "ASSIGNED": {  
            "i_pas": 0.0018251121  
        }  
    },  
    "Dendrites": {  
        "capacitance": {  
            "PARAMETER": {  
                "cm": 0.8  
            }  
        },  
        "pas": {  
            "PARAMETER": {  
                "g_pas": 8.9686098654  
            },  
            "ASSIGNED": {  
                "i_pas": 0.0018251121  
            }  
        }  
    },  
    "Axon": {  
        "capacitance": {},  
        "pas": {  
            "PARAMETER": {  
                "g_pas": 7.1428571428  
            },  
            "ASSIGNED": {  
                "i_pas": 2.4999999999  
            }  
        }  
    },  
    "Spine Neck": {  
        "capacitance": {},  
        "pas": {  
            "PARAMETER": {  
                "g_pas": 7.1428571428  
            },  
            "ASSIGNED": {  
                "i_pas": 2.4999999999  
            }  
        }  
    }  
}
```

Import Functionality

The imported text file presents a straightforward structure that allows users to comprehend and modify the mechanisms' parameter values easily. This flexibility enables users to customise and adapt the parameters as needed. Moreover, the software facilitates the import of already modified text files, streamlining the database creation process for sets of mechanisms about a specific cell type.

With this robust feature, users can effortlessly establish a comprehensive database of mechanism sets associated with cell types, enhancing the software's utility and facilitating in-depth analyses.

Export cell model.

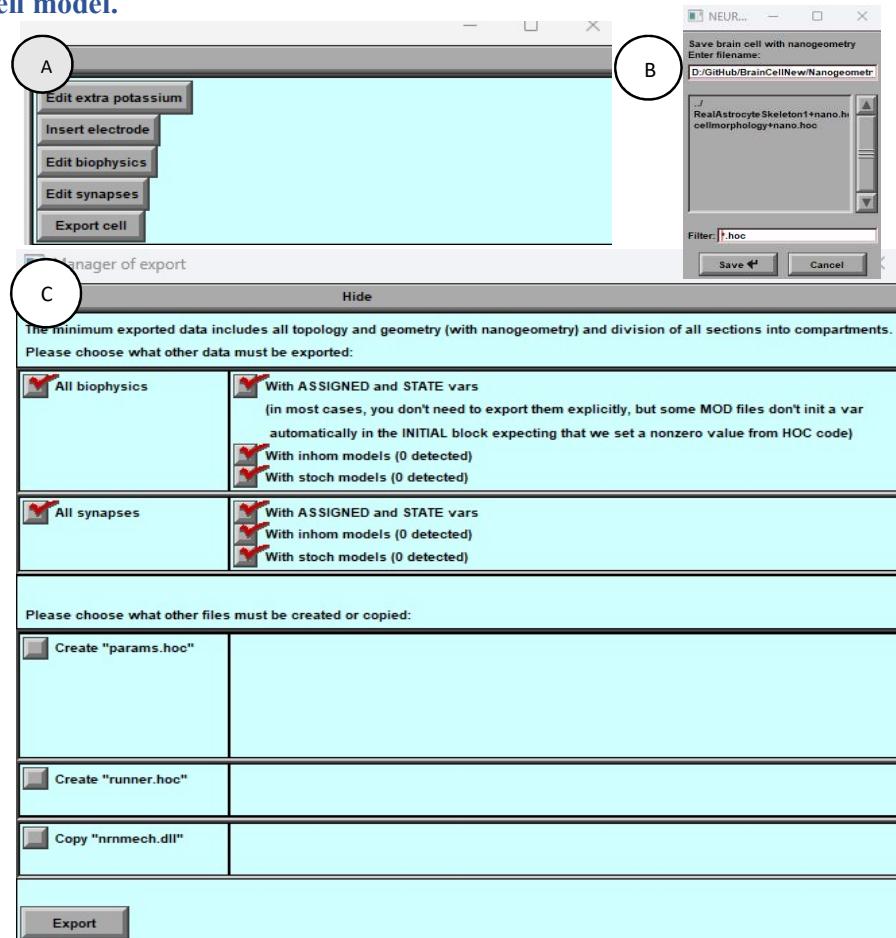


Figure 23. Control Panel for Exporting Finished Cell Model.

The Control Panel for Exporting Finished Cell Models is a crucial component of the BrainCell, providing users with comprehensive options for exporting their completed cell models. This panel offers convenient access to various export functionalities, facilitating the seamless transition of cell models to external platforms or further analysis.

A) Key to Call the Model Export Panel: The Control Panel features a designated key that users can activate to open the Model Export Panel. This key is a quick and intuitive way to access the export functionalities.

B) Pop-up Export Panel: Upon activation, the Model Export Panel appears as a pop-up window, displaying a range of export options and settings. This intuitive interface allows Users to navigate and configure their desired export parameters conveniently.

Model Export Panel

“Cell Shape”: Users can export the intricate shape and morphology of the cell model, ensuring accurate representation and preservation of its structural details.

“Biophysical Mechanisms”: The panel allows users to export the biophysical mechanisms incorporated within the cell model. This property includes all developed details, encompassing spatial heterogeneity and stochastic properties, ensuring the faithful representation of the model's behaviour.

“Synapses”: Users can export the synapses in the cell model, capturing all relevant parameters and spatial-stochastic properties. This feature enables the retention of crucial synaptic properties during the export process.

“Hock File Export”: The panel provides an option to export the cell model as a hock file, facilitating compatibility with other software or platforms that support this format. This ensures seamless integration with external tools and analysis pipelines.

“Cluster Calculation and Parameter Selector”: For advanced users working with cluster computing, the panel offers a dedicated control panel for further calculations on the cluster. It also includes a parameter selector that can be automatically adjusted during cluster calculations, enhancing efficiency and flexibility.

“File Export for Biophysical Mechanisms”: The panel allows users to generate an F file specific to the biophysical mechanisms employed in the cell model. This file serves as a convenient reference and can be used for various analyses and simulations involving the model's biophysical properties.

Users can easily export their cell models with the Control Panel for Exporting Finished Cell Models, preserving critical features such as cell shape, biophysical mechanisms, synapses, and more. This comprehensive export functionality empowers researchers to seamlessly collaborate, integrate, and analyse their cell models across different platforms and computational environments.

Structure of export file.

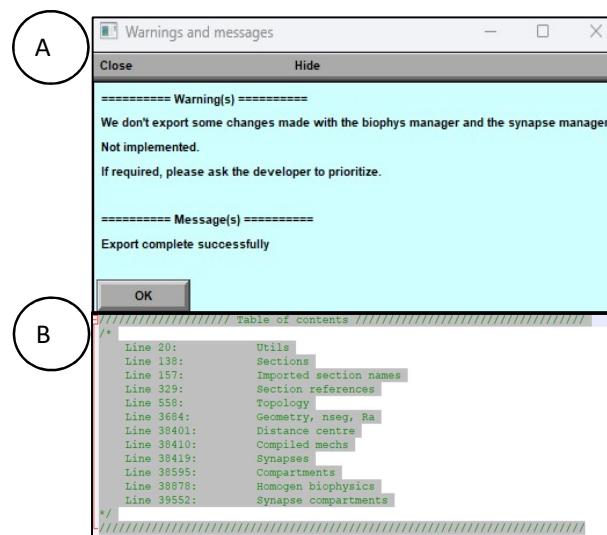


Figure 24. Form and Structure of the Export File. The panel displays warm messages (A) details about the format and arrangement of the export file (B).

Whenever the user exports a cell model, a notification window (Fig.20) will warn them of any errors or inaccuracies that may have occurred during the exporting process. It is important to note that these errors are usually minor and do not impact the further utilisation of the model. However, they serve as alerts for possible limitations within the model.

A successfully saved file in "hoc" - format will display inside as a commentary (Fig.20) a record of the mechanisms and their respective lines saved to the file. This valuable information facilitates efficient use of the model's features in subsequent calculations or when modifying the model.

BrainCell Software: Model Export. Result of Simulation Parameter Selection

Model Export Feature

1. Introduction The model export feature in BrainCell serves two primary purposes: saving specific parameters and lengthy calculations without external graphics or additional features provided by the software package. To effectively perform calculations, it is essential to specify which parameters will change during the calculation process.

2. Creating a Parameter File

2.1 Click the "Create File" button (Fig.24A) to initiate the process.

2.2 An additional window (Fig.24B) will open, allowing you to define the parameters that will be saved.

2.3 If you select "Export Other Parameters," a new window (Fig.24C) will appear to facilitate this process.

3. Export Parameters Window The Export Parameters window (Fig.24C) comprises two sections: a central panel for visualising the selected parameters and a left panel with five buttons for parameter manipulation.

3.1 Buttons in the Export Parameters Window:

"Add Biophys var."

- Clicking this button opens a pop-up window where you can select a biophysics parameter.
- The selected parameter will be displayed in the main window.

"Add synapse var."

- This button allows you to select a synapse parameter from a pop-up window.
- The chosen synapse parameter will appear in the main window.

"Add custom var."

- Use this button to select a parameter you created that may not be visible on the main Neuron panel.
- After selecting this option, the chosen parameter will appear in the main window.

"Correct var."

- If necessary, this button enables you to modify a specific parameter.

"Delete var."

- This button allows you to remove a specific parameter from the selection.

4. Saving Parameters Once you have made your parameter selections, click the "OK" button to save these parameters in the parameter file. By saving the parameters, you can perform further calculations while only modifying the saved parameters, streamlining the calculation process.

5. Performing Calculations Users can easily save, modify, and perform calculations in BrainCell by utilising the model export functionality and selecting the desired parameters.

Note: Refer to the figures (Fig.2A, Fig.24B, Fig.24C) for visual guidance throughout the process.

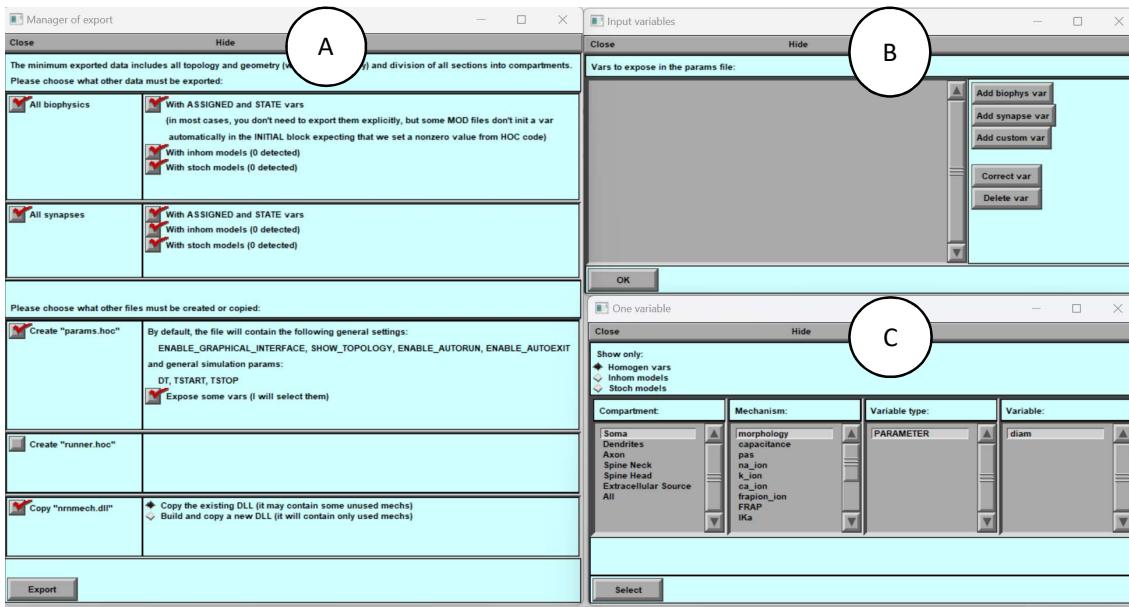


Figure 25. Exporting Control Parameters using Pop-up Windows

BrainCell allows users to define and export control parameters using pop-up windows. A. The first window saves specific parameters. B) the second window provides a selection interface for customising the output file. C) the third window gives an example of selecting soma diameter as a parameter. Users can easily create and export parameters for tailored simulations and analyses with these pop-up windows.

Exporting Computation Run parameters.

BrainCell allows users to export the results of their calculations by creating a "Result file" that contains specific variables of interest. This section explains how to export and record these variables during calculation.

1. Creating the "Runner. hoc" (Fig.26A):

- Press the "**“Runner. hoc”**" button to initiate the creation of the result file. The file will be named according to the time when the calculation is completed.
- This step readies the system to record and save the desired variables in the designated file.

2. Recording and Saving Variables:

- Press the "**“Record and save some vars”**" button, which opens a graph window (Fig.26B).

To record desired variables during the calculation, users can use the mouse to place the cursor on the graph window and utilise the standard neuron's "Print what?" function, as shown in **Figures 26B and C**.

- The selected variables will be saved to a file as a function of time, providing valuable data for further analysis.

3. Performing Calculations on a Parameter Grid:

- Utilize the "Make a sweep for some vars" button (Fig.26 D) to perform calculations on a parameter grid.

- Unlike previous methods allowing only one input parameter, this functionality enables users to create a set of input parameters for automated calculations.

4. Configuring the Parameter Grid (Fig.26E):

- A pop-up window will appear, allowing users to define the parameters of the grid.

- Set the initial and final values of the desired parameter and the step between them.

- Additionally, the pop-up window allows choosing between a linear or non-linear grid configuration.

Following these steps, users can export computation results by creating a "Result file" with specific variables of interest. The window (Fig.26D) facilitates the selection and recording of variables, while the parameter grid functionality enables automated calculations on a range of input parameters. This comprehensive approach enhances analysis capabilities and allows a more comprehensive exploration of model behaviour.

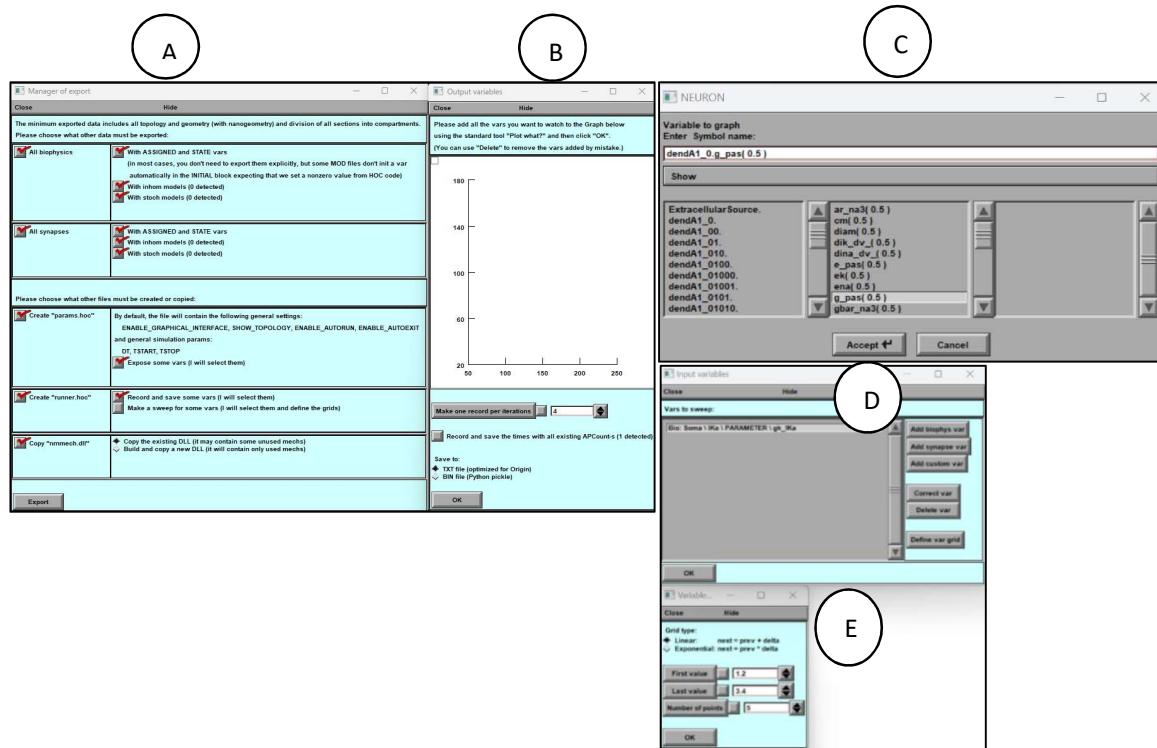


Figure 26. Exporting of control of computation Pop-up Windows. BrainCell allows users to define and export the control of computation pop-up windows. A. The first window saves specific parameters. B) the second window provides a plot for customising the output file. C) the third window gives an example of selecting somatic gpas as a variable in the result file. D) window gives an example of selecting soma gk as a parameter. F) the window for the grid definition.

Managing Computation Results

This section explains how to export cell models, ensuring easy access to all relevant properties and computation findings.

The well-organized directory (Fig.27) structure allows users to navigate easily and access exported cell models and their corresponding simulation results. This structure promotes efficient management and retrieval of relevant files, enabling in-depth analysis and exploration of cell models and computation outcomes. Below are step-by-step instructions on how to export the cell model.

Creating a Separate Folder for Cell Models:

- The BrainCell allows you to make a distinct folder for a particular cell when exporting a cell model.
- To maintain organisation and provide quick access to specific models, making a new folder for each exported cell is advised.

Folder Structure and Contents:

- All the files required for the cell model can be found inside the newly formed folder.
- The folder will include information about the cell's geometry, biophysical mechanisms, control files, and computation-related factors.
- Users may refer to and understand how specific results were obtained and under what conditions by organising cell models in this way.
- The essential feature of the folder is that it keeps the calculation results linked with the particular cell model.

Loading Cell Files and Inspecting Cell Configuration:

- A significant feature of these exported files is importing them back into the BrainCell software. Uploading the cell model file allows users to thoroughly inspect the entire cell configuration, including its properties and associated computation results.
- This feature enhances the user's ability to analyse and understand the intricacies of cell models, facilitating comprehensive examination and investigation.

By following these guidelines, users can effectively manage and organise cell models and computation results in the BrainCell software. Creating separate folders for each exported cell ensures easy access to relevant files and allows for a detailed examination of the cell's configuration and associated findings.

d:\GitHub\BrainCellNew\Nanogeometry\Neuron*.*			
Name	Ext	Size	Date
[..]	<DIR>		19/06/2023 12:25
[Cell1]	<DIR>		20/06/2023 10:06
[Cell2]	<DIR>		20/06/2023 10:57
cellmorphology+nano	hoc	2,441,843	19/06/2023 12:28
drag&drop_init	bat	42	18/06/2023 15:50
nrmec	dll	597,146	18/06/2023 15:50
d:\GitHub\BrainCellNew\Nanogeometry\Neuron\Cell1*.*			
Name	Ext	Size	Date
[..]	<DIR>		20/06/2023 10:50
[results]	<DIR>		20/06/2023 10:06
nrmec	dll	597,146	20/06/2023 09:57
runner	hoc	12,266	20/06/2023 09:57
params	hoc	255	20/06/2023 09:57
Cell1	hoc	14,082,988	20/06/2023 09:57
d:\GitHub\BrainCellNew\Nanogeometry\Neuron\Cell1\results*.*			
Name	Ext	Size	Date
[..]	<DIR>		20/06/2023 10:06
aps 2023-06-20 10.06.15	txt	111	20/06/2023 10:06
vars 2023-06-20 10.06.15	txt	19,135	20/06/2023 10:06
aps 2023-06-20 10.04.31	txt	111	20/06/2023 10:04
vars 2023-06-20 10.04.31	txt	19,135	20/06/2023 10:04
aps 2023-06-20 10.02.47	txt	111	20/06/2023 10:02
vars 2023-06-20 10.02.47	txt	19,135	20/06/2023 10:02
aps 2023-06-20 10.01.02	txt	111	20/06/2023 10:01
vars 2023-06-20 10.01.02	txt	19,135	20/06/2023 10:01
aps 2023-06-20 09.59.18	txt	111	20/06/2023 09:59
vars 2023-06-20 09.59.18	txt	19,135	20/06/2023 09:59

Figure 27. Organisational Structure of Imported Cell Models and Simulation Results

Overview: This figure demonstrates the directory structure export of BrainCell software, showcasing imported cell models and computer simulation results.

A) Initial Directory View:

Two directories, "Cell1" and "Cell2," house cell models and related files in the "Neuron/NanoGeometry" subdirectory.

B) Structure of Cell1 Directory:

The directory structure for the "Cell1" folder is displayed in this window. This structure remains consistent for all saved models.

- "exported cell1" - the exported cell model file.
- "nrmec.dll" - a mechanism file containing relevant computational mechanisms.
- "Runner.hoc" - a calculation management file responsible for simulations.
- "Params.hoc" - a file housing parameters modifiable during computation.

C) Results Directory:

Within this directory, various result files showcase computation outcomes for different cell models. In this instance, ten result files correspond to five calculation parameters. The first file illustrates action potential (AP) dynamics, while the second file (VARS) displays selected variable dynamics during the cell export process.

Re-import cell model.

The "Nano" option (Fig.2) allows reimporting the model into BrainCell.

To re-import a cell model, first access the BrainCell software. Then, select the Nano option (Fig.2) and load the desired cell model. With this selected cell model, the user can access all parameters and configurations and modify these parameters to perform new calculations and research. Remember to export to a new folder to avoid overwriting existing results.

Import different cell models from the Neuron Database.

Experimental Version for Model Import

This feature allows the integration of external models into the brain cell system, allowing for the convenient analysis, modification, and expansion of neural simulations. This experimental feature may be subject to updates and refinements in future versions.

This feature is currently in experimental development and is subject to ongoing updates. It enables users to load existing models of various cells written in the NEURON language, provided they contain at least one soma and one dendrite. Here's how to utilise this option:

1. Compile MOD Files:

- Before importing, the user must combine and compile the MOD files from the Brain Cell program into a single library, and the model files must be imported. Before calling the “mknrndll” utility, ensure these files are in the same directory. Place the generated DLL (Dynamic Link Library) file in the same directory as the simulation entry point HOC file.

2. Importing the Model:

- The user can import the main source file (typically **init.hoc**) using the "**External Simulation**" option.
- During the import process, calculations may start immediately, but you can pause them and continue the import.

3. Defining Cell Components:

- At this stage, BrainCell prompts to specify which components represent dendrites, soma, and axons within the imported model.

4. Post-Import Modifications:

- After a successful import, the user can rename or redefine all sections of the model as needed.

5. Biophysical Mechanisms and Export:

- Once the download is complete, the user can review all biophysical mechanisms, rename them if required, and export the cell.
- The user now has a model that can be seamlessly imported into the Brain Cell system for further calculations and modifications.

Simulation modes: Examples of cell simulation inside of BrainCell.

Various computational scenarios are available in BrainCell, all conveniently located on the right side of the main program panel.

1. Voltage
 - [Membrane voltage distribution](#)
 - [CA1-neuron voltage \(Neuron mode only\)](#)
2. Electric stimuli
 - [Constant electric stimuli](#)
 - [Variable electric stimuli](#)
3. FRAP
 - FRAP with round spot bleaching
 - [Line-scan FRAP](#)
4. Calcium
 - [Microscopic calcium dynamics \(Astrocyte mode only\)](#)
 - [Calcium waves](#)
5. Other Species
 - [Membrane biophysics with glutamate transport](#)
 - [Dynamic of intra and extracellular potassium](#)
 - [Basket cell GABA diffusion \(Neuron mode only\)](#)

While sharing some foundational similarities, these scenarios exhibit unique features tailored to astrocytes and neurons. For astrocytes, there is a specific scenario designed to simulate the dynamics of spontaneous calcium responses. For neurons, a dedicated scenario focuses on modeling the dynamics of glutamate release from an array of basket cell terminals.

The following sections provide a detailed breakdown of each scenario, enabling straightforward replication in computational studies. These scenarios are not merely instructional; they serve as versatile source packages capable of simulating a variety of physiological tasks specifically designed to address diverse research objectives.

CA1-neuron voltage (Neuron mode only)

CA1 pyramidal cells (PCs) provide a significant output of the hippocampus proper. They integrate information from the entorhinal cortex via the temporoammonic pathway and indirectly via the polysynaptic dentate gyrus–CA3–CA1 loop.

The dendrites of CA1 pyramidal neurons in the hippocampus express numerous types of voltage-gated ion channels, but the distributions or densities of many of these channels are very non-uniform. Sodium channels in the dendrites are responsible for action potential (AP) propagation from the axon into the dendrites (back-propagation); calcium channels are responsible for local changes in dendritic calcium concentrations following back-propagating APs and synaptic potentials; and potassium channels help regulate overall dendritic excitability.

Action Potential Dynamics Calculation

This feature enables dynamic calculations of action potentials within a cell based on the loaded shape and the synapses defined on its spines. Note the following considerations:

- **Cell Shape:** The calculations are based on the loaded cell shape, which may not accurately replicate a real pyramidal cell but serves as the framework for the simulation.
- **Synapse Definition:** The defined synapses on the spines are incorporated into the action potential dynamics calculations.

Certain parameters are fixed during the simulation:

- **Ion Channel Kinetics:** The ion channel kinetics within the cell follow a classical distribution predefined in the model. Detailed information about this distribution can be found in Migliore et al. (1999). For further details, refer to the linked model documentation.

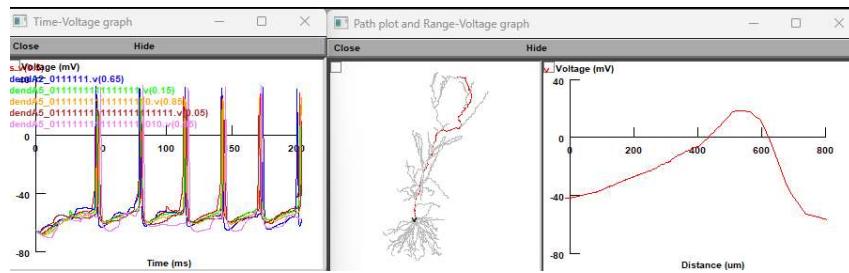
This feature allows for in-depth exploration of action potential dynamics in the context of the loaded cell shape and specified synapses, offering valuable insights into neural activity.

Adapting Cell Sections

If the names of sections (e.g., dendrites, soma, axon) in the loaded 3D cell structure differ from typical pyramidal cell features, BrainCell will display a warning. Should the warning be ignored, BrainCell will automatically assign the required mechanisms to sections identified as dendrites, soma, or axons.

Sections can be divided and renamed based on program recommendations to ensure the cell geometry meets simulation requirements. This process ensures full compatibility with the desired cell configuration, providing flexibility to adapt the cell structure while maintaining a seamless simulation experience.

Visualising Electrical Dynamics



Three distinct graphs visually represent the cell's electrical dynamics. The first graph offers insight into the dynamics across various cell sections along a spatial path. This spatial path corresponds to the illustration (in red) provided in the following figure and depicts the potential dynamics along this path. This graphical representation lets you observe and analyse the cell's electrical behaviour.

Membrane voltage distribution

In the main control panel, pressing '**Compute the spatial voltage distribution**' opens window panels which report membrane voltage across selected cell processes upon a step current injection at the soma (a common electrophysiological test scenario). The default resting potential is -85 mV.

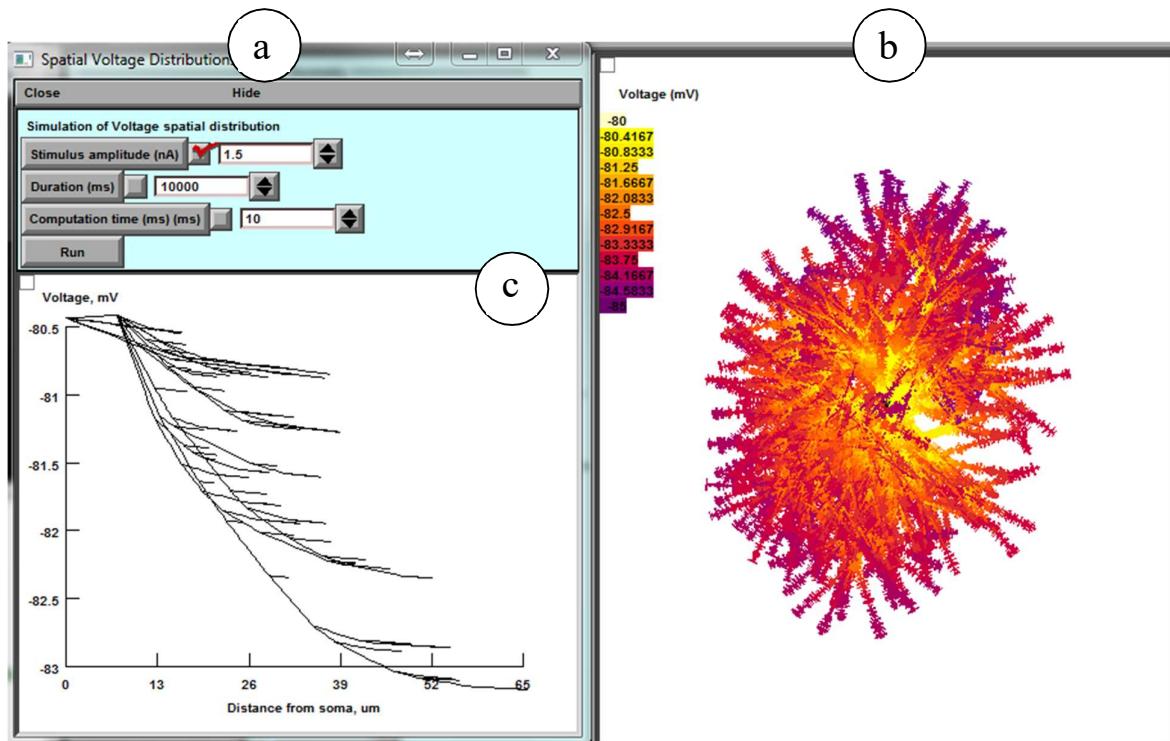


Figure 28. Window panel to monitor membrane space voltage distribution. **A**, Settings panel with space profile of voltage after inserting 1.5 nA into the soma. **b**, Dynamic voltage landscape mapped on the cell morphology. **c**, The corresponding voltage profile plot along individual processes ('dendrites' in NEURON).

The initiation panel (Fig. 28a) provides the following settings:

'**Stimulus amplitude (nA)**', the amplitude of constant depolarising current injected into the soma. '**Duration (ms)**', duration of constant depolarising current.

'**Computation time (ms)**', simulation run time (time of display).

'**Run**' to start the simulation.

Exploring Constant Electrical Stimulation

The "Constant Electrical Stimulation" tool lets to explore the dynamic interplay between current and voltage, unlocking the secrets of electrical signalling in digital neurons.

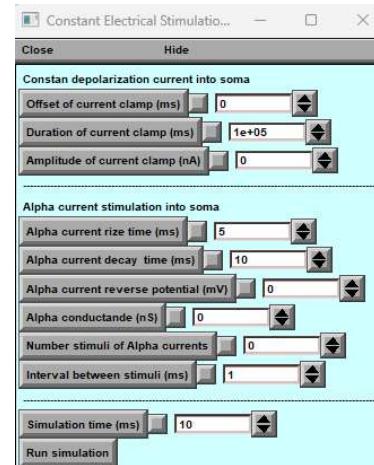
Directly Stimulate the Soma:

Bypass synaptic complexities and focus on the core of the neuron – the soma. Inject a steady current stream directly into the soma, observing its immediate and sustained impact on the membrane potential—three parameters: Offset, Duration and Amplitude.

Simulation of Alpha Synapses:

Activate alpha synapses on the soma and witness the interplay between direct and indirect stimulation. See how the neuron integrates these inputs, revealing the intricate dance of voltage fluctuations - 6 parameters: rise and decay time, reverse conductance number of stimuli and interval between stimuli. Potential.

The dedicated panel makes it easy to access current-clamp mode, allowing for quick configuration of stimulation parameters and experimentation. This demo serves as a starting point for exploring complex neurophysiological phenomena. Adjust parameters, try out different configurations, and better understand how neurons react to electrical stimuli.



Exploring Variable Electrical Stimulation

The "Variable Electrical Stimulation" tool lets to explore the sinus curve injected into the soma. Discover membrane potential dynamics with this feature! Experiment with alternating current and learn about voltage fluctuations within neurons.

Explore demo mode:

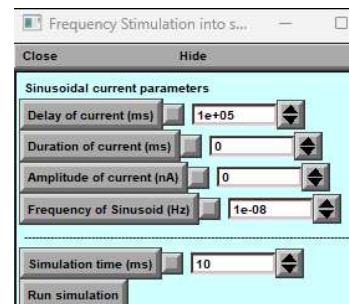
- Dedicated Panel: Input the periodic signal frequency and observe the dynamic response in current clamp mode.

- Sinusoidal Playground: Experiment with frequency range and analyse the relationship between stimulus and response.

- Current Clamp Magic: Inject current into soma and mimic real-world effects of electrical stimulation on a neuron.

"Variable Electrical Stimulation" is a powerful tool that can help:

- Visualize action potential generation.
- Investigate membrane properties.
- Design custom electrical stimulation protocols.



Remember: As you explore, remember that the specific responses you observe will depend on the chosen cell model and its unique biophysical properties. Don't hesitate to experiment with different models and parameters to unlock the full potential of this versatile tool.

Linescan FRAP experiment: probing intracellular connectivity of Brain Cell

In the "Fluorescence Recovery After Photobleaching (FRAP) for Intracellular Indicators" section of the BrainCell software manual, we show how FRAP can evaluate effective diffusivity in cellular compartments near the bleached area.

FRAP is particularly useful in verifying if the modelled cell morphology accurately replicates empirically documented intracellular diffusivity and connectivity. The BrainCell software enables users to conduct FRAP experiments and corresponding simulations directly from the main window, providing a practical tool to test astrocyte morphology against empirical data.

The software's default settings are based on data from CA1 astrocyte studies using linear photobleaching tests with Alexa Fluor 594 or Alexa Fluor 488. The FRAP test in BrainCell simulates a photobleaching area represented by a linear segment on the XY plane. Select 'Line-scan FRAP' to access this function, which opens a dedicated control window (refer to Fig. 24 in the manual).

The initiation panel of 'Line-scan FRAP' (Fig. 29a) includes several adjustable settings:

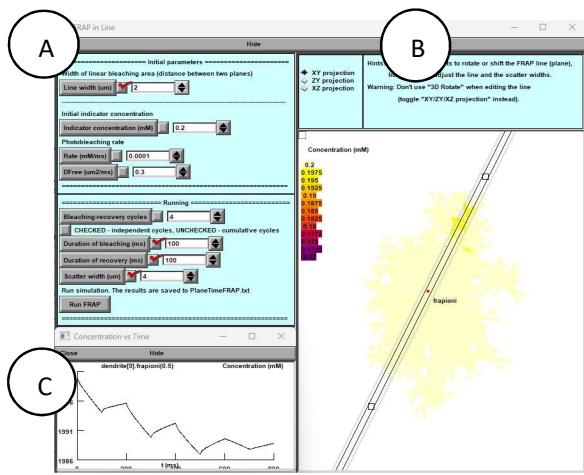


Figure 29. Window panels to control and monitor simulated line-scan FRAP. A, Parameter settings panel. B, The Concentration dynamics of non-bleached molecules are colour-coded and mapped on cell morphology. C, Concentration time profile sample.

The default duration of FRAP trials is 8000 ms, with photobleaching occurring every 1000 ms. In addition to the colour-coded shape map display (Fig. 29b), the results of FRAP simulations could be plotted and saved using standard NEURON functions: for instance, by selecting and identifying the cell branch/area of interest ('dendrite' in NEURON format) using **PointProcessGroupManager**, and next configuring the output display and store using the **Graph** menu (Fig. 29c).

After the end of stimulation, the software automatically saves the data file of the results.

RAP Panel

- **Width of linear bleaching area (initial distance between two planes):** Sets the width of the bleaching line in micrometres.
- **Line width (μm):** Sets the width of the bleaching line in micrometres.
- **Initial indicator concentration (mM):** Sets the initial concentration of the fluorescent indicator in the cell.
- **Photobleaching rate (ms):** Sets the rate at which the indicator is bleached by the laser.
- **Diffusion rate (μm²/ms):** Sets the diffusion rate of the indicator in the cell.

Bleaching Recovery Cycles

- **CHECKED (independent cycles), UNCHECKED (cumulative cycles):** Determines whether each cycle is independent or cumulative.
- **Duration of bleaching (ms):** Sets the duration of the bleaching pulse.
- **Duration of recovery (ms):** Sets the duration of the recovery period after each bleaching pulse.

Run FRAP

- **Run simulation. The results are saved to Plane1/mFRAP.txt:** Starts the FRAP simulation.

Hints

- **Drag '3 anchor points' to rotate and shift the FRAP line (plane).**
- **Hold 'shift' to adjust the line or the scatter widths.**
- **Don't use '3D Rotate' when editing the line.**
- **Toggle 'XY/YZ projection' instead.**
- **Concentration (mM):** Shows the concentration of the fluorescent indicator in the cell.

Concentration vs Time: Shows the concentration of the fluorescent indicator over time.

- **XY Projection:** Shows the 2D projection of the FRAP line and the fluorescent indicator distribution in the XY plane.
- **YZ Projection:** Shows the 2D projection of the FRAP line and the fluorescent indicator distribution in the YZ plane.
- **XZ Projection:** Shows the 2D projection of the FRAP line and the fluorescent indicator distribution in the XZ plane.

Notes

- The FRAP line can be rotated and shifted using the three anchor points.
- The width of the FRAP line and the scatter widths can be adjusted using the shift key.
- The 3D rotation function should not be used when editing the FRAP line.
- The XY/YZ projection toggle button can switch between the XY and YZ projections.

Additional Notes

- The FRAP simulation can be used to model the diffusion of fluorescent indicators in cells.
- The FRAP line can be used to bleach a specific region of the cell.
- The fluorescence recovery after bleaching can be used to measure the diffusion rate of the indicator.

FRAP with round spot bleaching

In this section, we introduce a user-friendly computational scenario designed for users of all skill levels, including non-specialists. This scenario demonstrates how BrainCell can be utilized to study cell geometry through a process known as an FRAP (Fluorescence Recovery After Photobleaching) experiment. In simpler terms, FRAP involves using a laser to temporarily 'bleach' or dim a small, specific area within a cell to observe how it recovers.

BrainCell simplifies this process by allowing you to simulate a FRAP experiment on a virtual cell. During the simulation, you can focus on a particular region of the cell and observe how the fluorescence inside the spherical volume of a small radius - or the cell's natural glow under the microscope - returns after bleaching. The software not only enables you to run a single experiment but also allows you to create a series of these experiments. This is particularly useful for understanding how fluorescence recovery varies depending on the distance from the cell's centre. With BrainCell, you can quickly generate and analyse these results to better understand the cell's geometry and behaviour.

The software's default settings are based on data from CA1 astrocyte studies using linear photobleaching tests with Alexa Fluor 594 or Alexa Fluor 488. The FRAP test in BrainCell simulates a photobleaching area represented by a spherical segment on the different planes. Select 'Round spot FRAP' to access this function, which opens a dedicated control window (refer to Fig. 30 in the manual).

The initiation panel of 'Round spot FRAP' (Fig. 30a) includes several adjustable settings:

1. **Spot Radius:** This parameter determines the sphere's radius that represents the area affected by photobleaching. In two-photon excitation mode, the focused laser beam typically creates a point-spread function ranging from 1 to 1.5 μm , depending on the system's optics. You can adjust the spot radius to align with these values using your mouse on the plot, ensuring your simulation mirrors actual experimental conditions.
2. **Initial Concentration (mM):** This setting allows you to define the basal concentration of photo-bleachable dye molecules within the bleached zone after photobleaching. It is set to 0 by default, indicating that all molecules within the area are bleached initially.
3. **Time of Recovery:** This option lets you specify the duration of the recovery phase after photobleaching, allowing you to observe how quickly fluorescence returns to the bleached area.
4. **Number of Trials:** This parameter sets the number of recovery trials you wish to simulate at different locations within the cell. The locations for these trials are chosen randomly within the defined scatter radius.
5. **Scatter Radius:** This radius defines the spherical area within which recovery trials post-photobleaching is calculated, allowing you to observe how recovery varies across different cell regions.
6. **Diffusion Coefficient:** Set at 0.3 $\mu\text{m}^2/\text{ms}$, this coefficient represents the rate at which dye molecules diffuse through the cell, a crucial factor in understanding the dynamics of fluorescence recovery.

Through these settings, the BrainCell software offers a versatile and detailed approach to conducting and simulating FRAP experiments, providing valuable insights into cellular diffusivity and morphology.

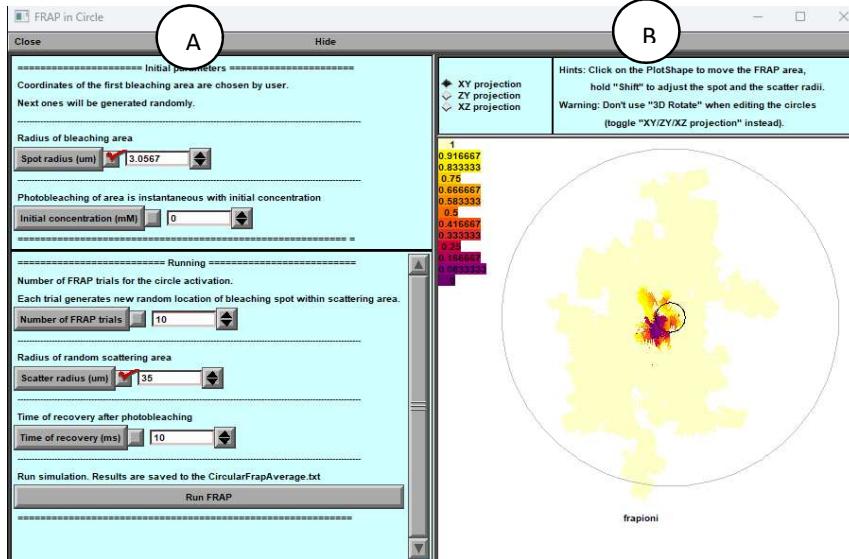


Figure 30. Window panels to control and monitor simulated spot FRAP.

A. Parameter Settings Panel: This panel is where you can adjust various settings for the FRAP simulation. Here, you can set parameters like the spot radius, initial concentration, time of recovery, number of trials, scatter radius, and diffusion coefficient. These settings allow you to customize the FRAP simulation according to your specific research needs or experimental design.

B. Concentration Dynamics Visualization: This part of the interface shows a color-coded, dynamic map of the concentration of non-bleached molecules on the cell's morphology. It provides a visual representation of how the concentration changes over time during the recovery process post-photobleaching, offering an intuitive understanding of the molecular behavior within the cell.

Ca²⁺ wave simulations

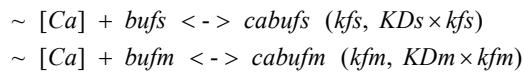
Pressing the 'Calcium wave' key on the main control panel opens new windows (Fig. 26) for setting, controlling, and displaying intracellular Ca²⁺ dynamics in response to a local increase in the Ca²⁺ channel ligand IP3 concentration.

BrainCell employs the standard NEURON-integrated mathematical formalism of Ca²⁺ reaction-diffusion kinetics, including buffering and removal, which has been tested and validated in numerous studies; its detailed description can be found in the **radius.mod** file. The basic set of equations of calcium dynamics is as follows (built-in NEURON functions and forms are used).

$\frac{d[Ca]}{dt} = D_{ca} \frac{d^2[Ca]}{dt^2} - \frac{\pi(i_{ca} - i_{pmp}) \times diam}{2 \times FARADAY}$, where [Ca] is Ca²⁺ concentration, D_{ca} - diffusion coefficient, diam is a local diameter, i_{ca} is a sum of voltage-dependent potassium currents, and the pump current i_{pump} is

$$i_{pmp} = 2 \frac{FARADAY \times (f_{flux} - b_{flux})}{area}, \text{ where } f_{flux} \text{ and } b_{flux} \text{ are the forward and reverse fluxes}$$

Two types of buffers are used, mobile "bufm" and endogenous "bufs" with corresponding kinetic constants kf and KD:



SERCA pump, channel, and leak with alpha, relative abundance of SERCA mechanism

$$\text{pump current : } \frac{d[Ca]}{dt} = (-v_{max} \times \frac{[Ca]^2}{[Ca]^2 + Kp^2})$$

channel : $hc \rightleftharpoons ho$ ($kon \times Kin_h$, $kon \times ca$), j_{chnl} is Ca flux releases from SERCA to cytoplasm

$$\frac{d[Ca]}{dt} = (\alpha \times j_{max} \times (1 - \frac{[Ca]}{caer}) \times (\frac{ip3i}{ip3i + Kp3} \times \frac{[Ca] \times ho}{ca + Kact})^3)$$

$$\text{leak : } \frac{d[Ca]}{dt} = (\alpha \times L \times (1 - \frac{[Ca]}{caer})), \quad L = v_{max} \times [Ca]^2 / \frac{[Ca]^2}{[Ca]^2 + Kp^2} / (1 - \frac{[Ca]}{caer})$$

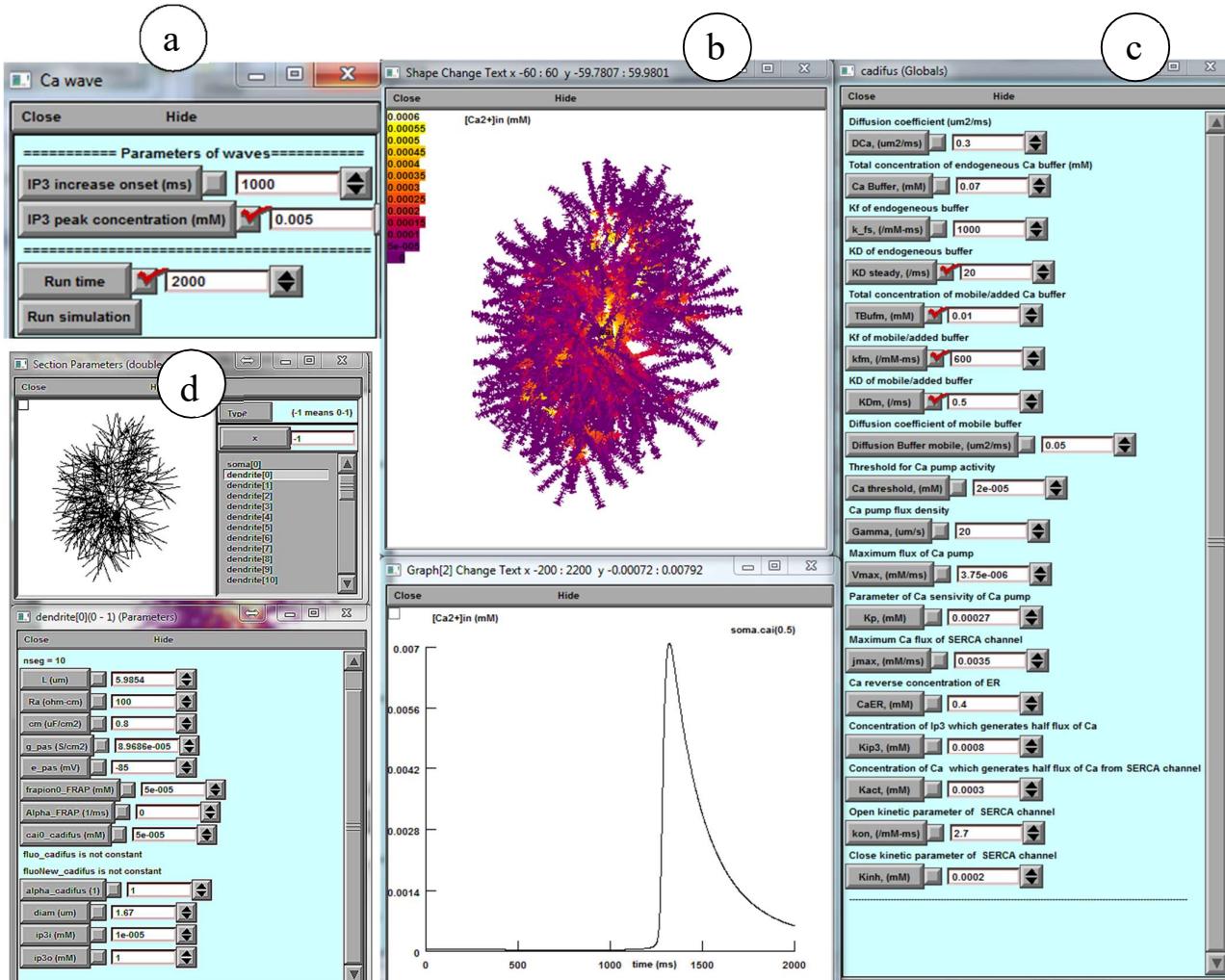


Figure 31. Control and Monitoring Panels for Ca²⁺ Wave Dynamics: a. Ca²⁺ Wave Trigger Settings Panel: Configure Ca²⁺ wave trigger settings. b. Dynamic [Ca²⁺] Landscape and Readout Plot: Top: Visualization of the dynamic [Ca²⁺] landscape colour-coded and mapped onto cell morphology. Bottom: Readout plot depicting [Ca²⁺] dynamics, focusing on the soma (default view). c. Parameter Setting Panel for Ca²⁺ Reaction-Diffusion Processes: Panel to set parameters related to Ca²⁺ reaction-diffusion processes. d. Ca²⁺ Homeostasis and Dynamics Monitoring Panel: Panel for monitoring Ca²⁺ homeostasis and dynamics at individual processes (referred to as 'dendrites' in NEURON nomenclature)

'Ca wave' panel (Fig. 30a) has controls as follows:

'IP₃ increase onset (ms)', sets the onset of a step increase in the IP₃ concentration;

'IP₃ peak concentration (mM)', sets amplitude (0.005 mM shown).

'Run time' sets simulation run time (default 2000 s).

The window parameter setting panel for Ca²⁺ reaction-diffusion (Fig. 31c) displays the corresponding explanations above the setting keys. The concentration and kinetic parameters of the Ca²⁺ indicator (Fluo-4) are constrained by experimental measurements, whereas the default values for Ca²⁺ pumps and channels correspond to estimates found in the literature. The user should further constrain these parameters based on specific experimental tests.

Once the current simulation run has ended, a new simulation run can start. The 'Graph' panel (Fig. 31b, bottom) provides the [Ca²⁺] time course in the soma. The current BrainCell version deals with IP₃ rises at the soma, but it can be adapted to 'release' IP₃ locally at any place in the astrocyte.

Simulating microscopic Ca^{2+} events

Pressing 'Calcium dynamics' key prompts four new windows (Fig. 31):

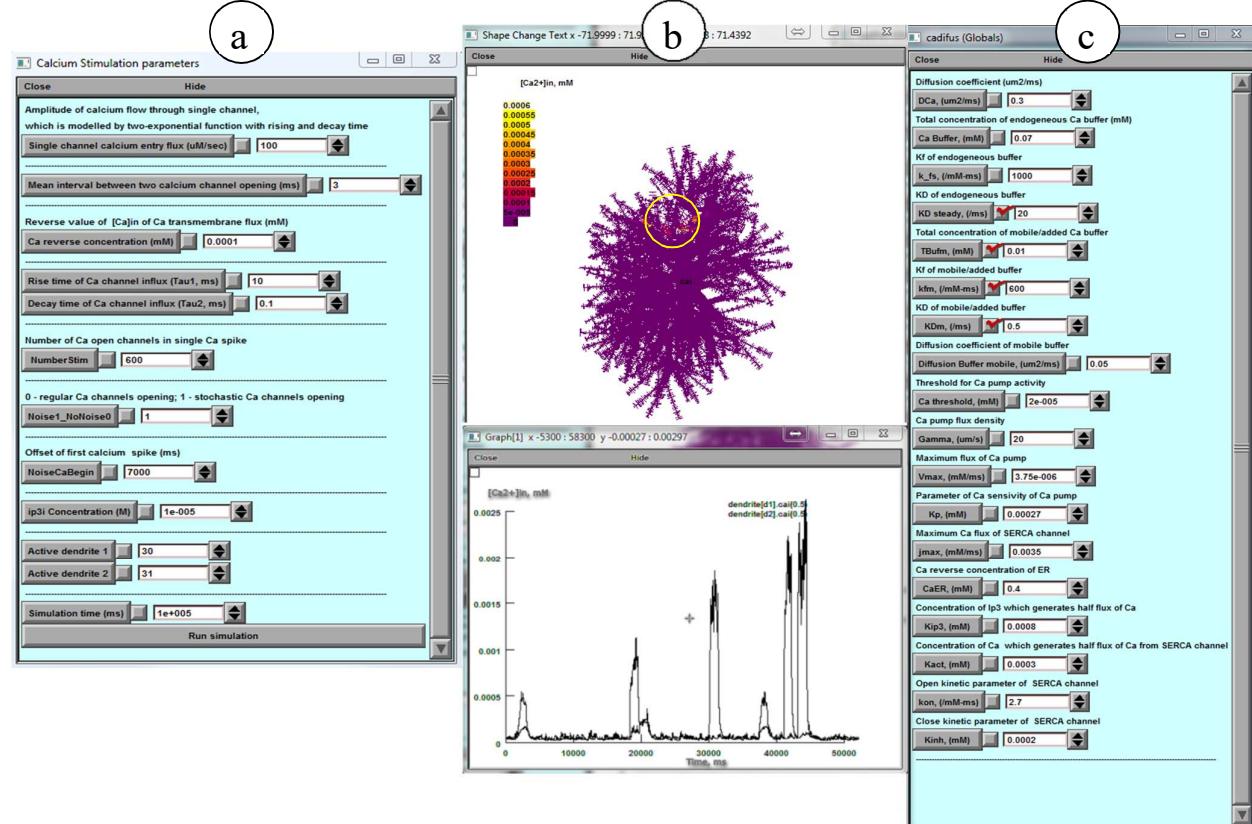


Figure 32. Window panels to control and monitor microscopic Ca^{2+} dynamics. **a**, Parameter settings for single-channel Ca^{2+} dynamics ('sparks' and 'puffs') in two selected 'active' branches (bottom: 'dendrite' numbers 30 and 31 are shown, in NEURON nomenclature). **b**, Visualisation of internal Ca^{2+} dynamics mapped on cell morphology (top), and $[\text{Ca}^{2+}]$ time course in two selected dendrites (bottom; dendrites d1 and d2 are shown); yellow circle depicts the area of interest ('dendrites' 30 and 31). **c**, Parameter setting panel for Ca^{2+} reaction-diffusion processes (as in Fig. 26c).

Calcium Stimulation Parameters Panel (Figure 31a):

The 'Calcium Stimulation Parameters' panel, depicted in Figure 27a, facilitates the adjustment of critical parameters governing microscopic Ca^{2+} entry kinetics. This entry process is hypothesized to comprise a sequence of stochastic Ca^{2+} channel openings encompassing higher-frequency bursts. Notably, many of these parameters still need to be discovered, emphasizing the need for ongoing investigative exploration and research.

- **'Single Channel Calcium Entry Flux':** Defines the rate of calcium entry during an individual event.
- **'Mean Interval Between Two Calcium Events':** Specifies the average time interval between consecutive events.
- **'Basal Ca^{2+} ':** Determines the basal $[\text{Ca}^{2+}]$, acting as the reverse concentration for the linear Ca^{2+} efflux.

- '**Rise' and 'Decay2+ flux during a single channel's opening.**
- '**Events per Burst
- '**Randomness
- '**Burst Onset
- '**IP3 Concentration
- '**Active Dendrite 1' and 'Active Dendrite 22+ entry is enabled.**
- '**Stimulation Time2+ dynamics are relatively slow, necessitating a simulation trial of at least 100 seconds to capture the intricacies of the process.**********

Utilizing this panel, users can tailor the simulation parameters to better comprehend and simulate the nuanced dynamics of calcium entry at a microscopic level.

Simulating glutamate transporters

Neuronal plasma membranes are enriched in high-affinity glutamate transporters which generate rapid inward current upon glutamate binding. In BrainCell main panel, pressing '**Glutamate transporters**' key opens the menu (Fig. 28), which enables simulations of the dynamic membrane voltage landscape mapped onto cell morphology, in response to volume-limited application ('uncaging') of glutamate at a selected area of the cell.

'Uncaging glutamate' panel (Fig. 28a, top) provides self-explanatory parameter settings for volume-limited glutamate application (uncaging) within a round area of the cell. The *glutamate transporter kinetics* (Bergels and Jahr, 1997 Neuron 19: 1297-1308) includes six independent states (C_i , where $i = 1, \dots, 6$). The detailed description can be found in the *GluTrans.mod* file. The basic relationships are

$$\begin{aligned}
 \frac{dC_1}{dt} &= C_1 [Glu]_o k_{12} u(v, -0.1) + C_2 k_{21} \text{ Parameters} \\
 \frac{dC_2}{dt} &= C_2 [Na]_o k_{23} u(v, 0.5) + C_3 k_{32} \quad k_{12} = 20 \quad (mM ms)^{-1} \\
 \frac{dC_3}{dt} &= C_3 k_{34} u(v, 0.4) + C_4 k_{43} \quad k_{23} = 0.015 \quad (mM ms)^{-1} \quad k_{21} = 0.1 \quad ms^{-1} \\
 \frac{dC_4}{dt} &= C_4 k_{45} + C_5 k_{54} [Glu]_{in} \quad k_{34} = 0.2 \quad ms^{-1} \quad k_{32} = 0.5 \quad ms^{-1} \\
 \frac{dC_5}{dt} &= C_5 k_{56} u(v, 0.6) + C_6 k_{65} [Na]_{in} \quad k_{45} = 4 \quad ms^{-1} \quad k_{43} = 0.6 \quad ms^{-1} \\
 \frac{dC_6}{dt} &= C_6 [K]_{in} k_{61} + C_1 k_{16} u(v, 0.6) [K]_o [K^+]_{in} = 120 \quad mM \quad k_{54} = 10 \quad (mM ms)^{-1} \\
 &\quad [Na^+]_{in} = 15 \quad mM \quad k_{65} = 0.1 \quad (mM ms)^{-1} \\
 &\quad [K^+]_o = 150 \quad mM \quad k_{61} = 2 \times 10^{-4} \quad (mM ms)^{-1} \\
 &\quad [Glu^+]_{in} = 0.3 \quad mM \quad [K^+]_o = 3 \quad mM \\
 &\quad [Glu^+]_o = 20 \times 10^{-6} \quad mM
 \end{aligned}$$

where $u(th, x) = \exp(th \times x / (2 \times 26.7))$

NOTE: To ensure stable membrane kinetics, the onset of uncaging must be >3 ms. The user can add another, simultaneously occurring uncaging spot, specifying the distance between the two spots at the distance X from the first one.

Transporter currents are computed in somatic voltage clamp configuration: the clamp parameters are indicated on the panel '**Somatic voltage clamp**' (Fig. 28b), including electrode resistance, clamp voltage and duration. The monitoring panel (Fig. 28c) displays a colour-coded membrane current landscape mapped onto cell morphology; the graph panel (Fig. 28d) displays the time course of extracellular glutamate and clamp current at selected cell processes.

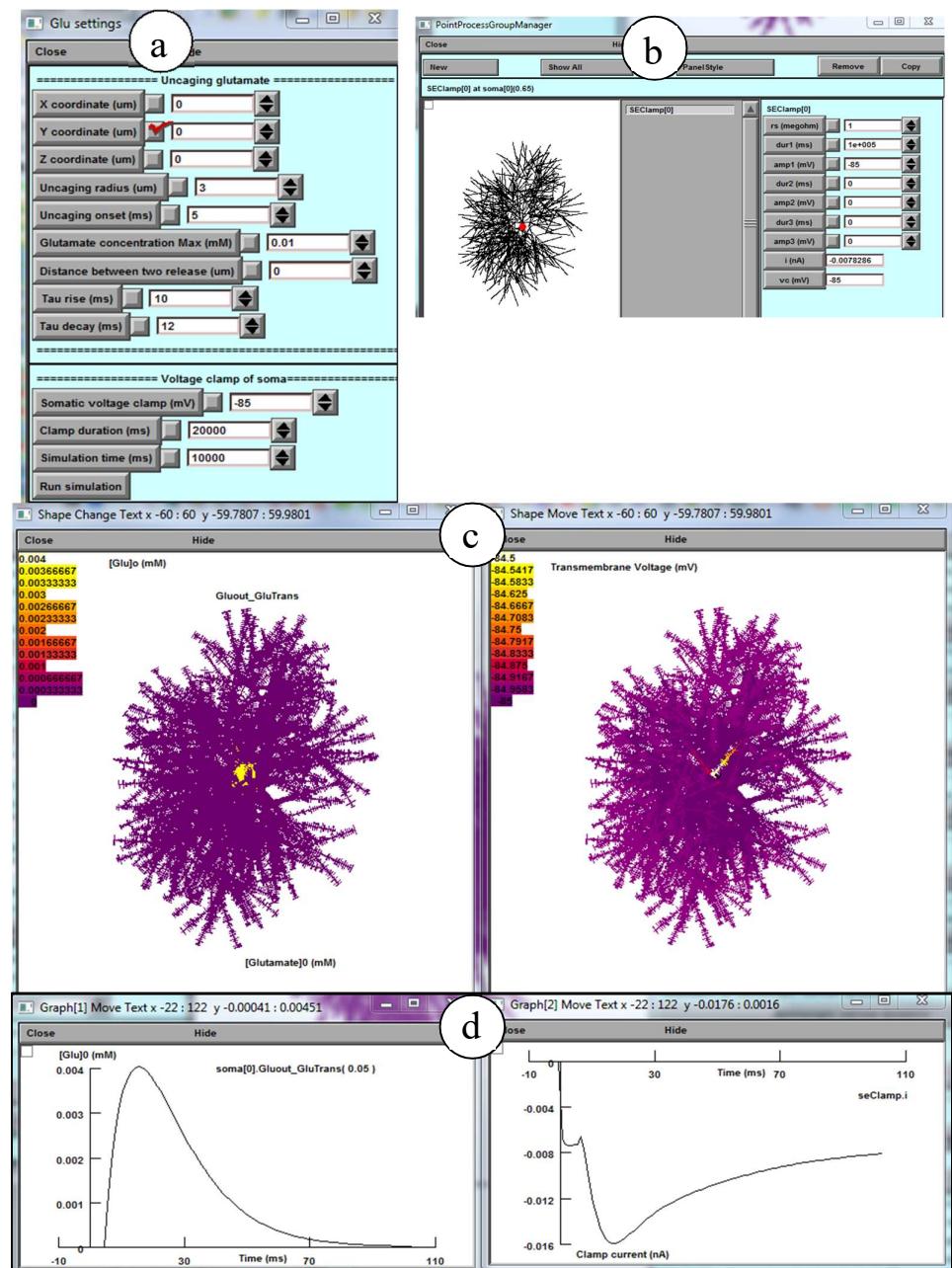


Figure 33. Window panels with parameter settings and readout plots simulate the dynamic membrane voltage landscape mapped onto cell morphology in response to volume-limited application ('uncaging') of glutamate. a, Glutamate uncaging parameter settings. b, Voltage-clamp parameter settings, including electrode positioning. c, Visualisation panel. d, Time course graph panel.

Window panel 'Glu setting' (Fig. 33a) provides parameters:

'X, Y and Z coordinate (um)' set respectively x, y and z-coordinates of the centre of the uncaging circle. 'Uncaging radius (um)' sets the radius of the uncaging circle.

'Uncaging onset (ms)' sets the onset of uncaging.

'Glutamate concentration Max (mM)' sets the maximum glutamate concentration during uncaging.

'Rise' and 'Decay tau (ms)' set the dynamics of glutamate uncaging.

Simulating potassium dynamics inside and outside cell

This option is to simulate the dynamics of intracellular $[K^+]$ resulting from local K^+ input and intracellular redistribution and efflux/leakage (Fig. 29). From the main panel of BrainCell, the user can activate the potassium dynamics by clicking the button '**Potassium Dynamics**' which prompts the relevant windows (Fig. 29).

A standard NEURON panel (Fig. 29a) is used to control the simulation and visualise the outcome. The '**Potassium settings**' panel (Fig. 29b) provides self-explanatory parameter settings for volume-limited potassium application (local current) within a round area of the cell. Here, $[K^+]$ dynamics are computed in somatic voltage clamp configuration: the clamp parameters are indicated on the panel '**Somatic voltage clamp**' (Fig. 29a, bottom), including electrode resistance, clamp voltage and duration

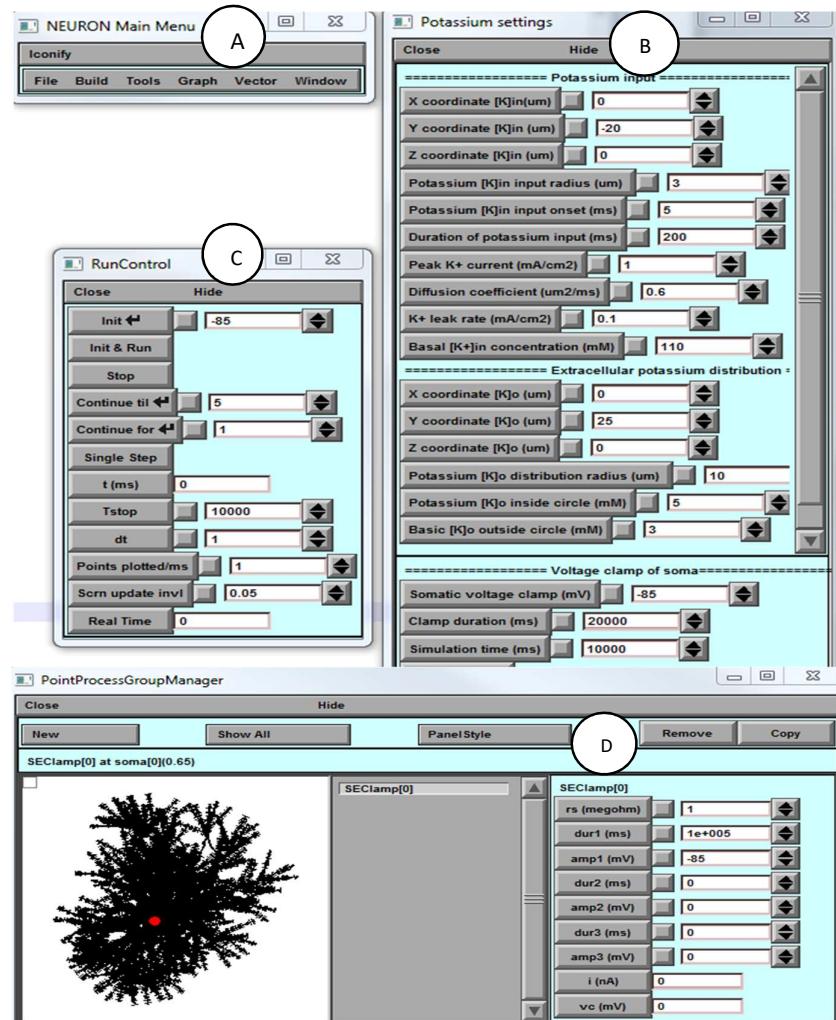


Figure 34. Window panels to control and monitor intracellular K^+ dynamics and extracellular K^+ -steady-state distribution. a, Parameter settings for monitoring $[K^+]_{in}$ and $[K^+]_{out}$ landscape mapped onto cell morphology. b, Parameter settings pertinent to volume-limited $[K^+]_{in}$ entry and $[K^+]_{out}$ distribution. c, Shape visualisation with voltage-clamp positioning. d, Somatic voltage clamp applied (bottom), for the sake of simplicity.

NOTE: The present simulation feature explores the diffusion landscape of intracellular K^+ while approximating the K^+ entry and extrusion kinetics with first-order reactions that are independent of membrane voltage, for the sake of clarity. A more detailed simulation paradigm could include further experimental constraints based on known (voltage- and concentration-dependent) K^+ pumps and channels, extracellular K^+ dynamics, and unclamped membrane voltage. Such membrane mechanisms, including astroglial $Kir4.1$ channels, are available through the standard NEURON

The monitoring panel (Fig. 35a) displays colour-coded $[K^+]_{in}$ concentration landscapes mapped onto cell morphology. The corresponding plot (Fig. 35c) shows the time course of intracellular $[K^+]_{in}$ at a selected location (dendrite [51]). The panel (Fig. 35b) shows the steady state distribution of extracellular $[K^+]$ onto cell morphology.

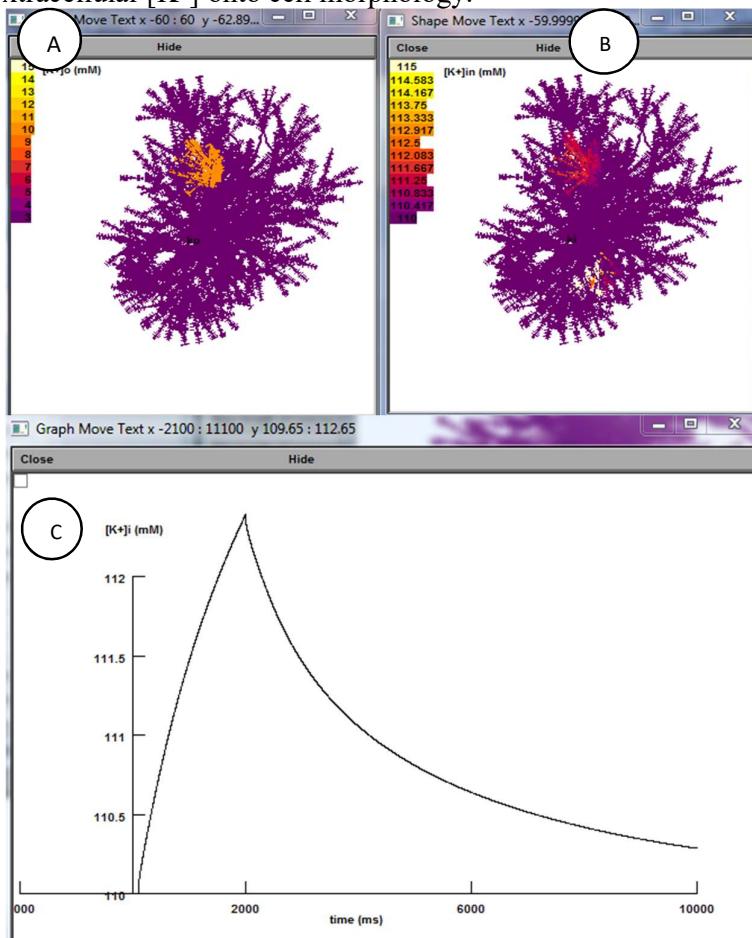


Figure 35. Window panels displaying simulated dynamics of intracellular $[K^+]_{in}$ and extracellular $[K^+]_o$ steady-state distribution mapped onto cell morphology (a) and (b), and $[K^+]_{in}$ in the time course (c) at a selected branch (dendrite), in response to volume-limited injection of potassium current (as in Fig. 34).

Window panel 'Potassium setting' provides parameter settings
 'X, Y and Z coordinate $[K]_{in}$ (um)' sets centroid co-coordinates for the K^+ entry area.
 'Potassium $[K]_{in}$ input radius (um)' sets the radius of K^+ entry area.
 'Potassium $[K]_{in}$ input onset (ms)' sets the onset of K^+ entry.
 'Duration of potassium input (ms)' sets the duration of K^+ entry. 'Peak K^+ current (mA/cm^2)' sets maximum amplitude of K^+ current entry.
 'Diffusion coefficient (mm^2/ms)' sets the intracellular K^+ diffusion coefficient.
 ' K^+ leak rate (mA/cm^2)' sets K_p , the K^+ extrusion current density.
 'Basal $[K^+]_{in}$ concentration (mM)' sets the resting intracellular $[K^+]$.

'Basal [K⁺]_{in} concentration (mM)' sets the basic potassium intracellular concentration.

Window panel "Extracellular potassium distribution"

'X, Y and Z coordinate [K]o (um) ' sets the centroid co-coordinates for the extracellular region of the [K⁺] fluctuation.

'Potassium [K]o distribution radius (um)' sets the radius of the extracellular K₊ region.

'Potassium [K]o inside circle (mM)' sets extracellular [K₊] inside the region.

'Basic [K]o outside circle (mM)' sets extracellular [K₊] outside the region.

Basket cell GABA diffusion (Neuron mode only)

This tutorial focused on creating and analyzing realistic basket cell neuron models. This tutorial is part of a series designed to guide you through building a neuron model and exploring its GABA (Gamma-Aminobutyric Acid) release dynamics. Here's what we'll cover:

1. **Creating GABA Synapses on Axon Terminal:** We will start by showing you how to create various numbers of GABA synapses located on the axon terminal of the neuron model. This step is crucial for understanding how synapse quantity can impact GABA release.
2. **Modifying Synaptic Release Function:** Next, we will explore how to alter the synaptic release function. This modification is essential for studying how different release mechanisms affect GABA diffusion.
3. **Including GABA Uptake:** An essential aspect of our tutorial is incorporating the process of GABA uptake. The uptake function will help you understand how GABA is reabsorbed and its effects on neuronal signalling.
4. **Designing GABA Concentration Dynamics Videos:** To visually represent the dynamics of GABA concentration, we will guide you through designing and creating videos. These videos will clearly and dynamically represent how GABA concentration changes over time.

This tutorial will identify the conditions under which a neuron can release GABA in regular and irregular patterns. This focus is significant because, in many instances, basket neurons release substantial amounts of GABA, leading to diffusion and uptake dynamics that are crucial for understanding neuronal communication and network behaviour.

The main panel window.

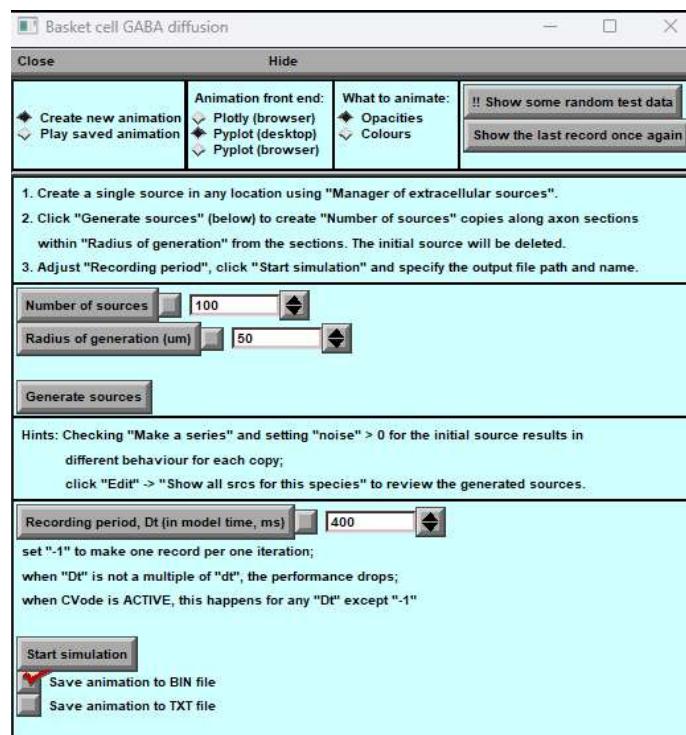


Figure 36. Source of the diffusion panel

Create new animation: Option to start a new animation sequence for the simulation.

Play saved animation: Option to play an animation that has been previously saved.

Plotly (browser): Toggle to use the Plotly graphing library in the browser for visualization.

Pyplot (desktop): Toggle to use the Pyplot library on the desktop for visualization.

Pyplot (browser): Toggle to use the Pyplot library in the browser for visualization.

What to Animate:

Opacities: Option to animate the opacity levels of the simulation elements.

Colours: Option to animate the color changes within the simulation.

Show some random test data: Option to display random test data in the animation.

Show the last record once again: Option to replay the last recorded simulation data.

Simulation Setup Instructions:

Create a single source in any location: Begin by creating a single source using the "Manager of extracellular sources".

Generate sources: Use this button to create a specified number of source copies distributed along axon sections within a set radius. The original source will be removed after this.

Adjust Recording period and Start simulation: Set the desired recording period for the simulation, then start the simulation process and designate the output file location and name.

Simulation Parameters:

Number of sources: Enter the total number of sources to generate in the simulation.

Radius of generation (μm): Specify the radius within which the sources should be generated around the axon sections.

Generate sources: Button to initiate the generation of sources based on the specified parameters.

Hints:

Make a series: Check this option to create a series of data points in the simulation.

Noise: Input a value greater than 0 to introduce variability in the behavior of each source.

Recording Period (Dt):

Enter the desired time interval for recording simulation data in model time (milliseconds).

Setting Dt to "-1" records data at every simulation iteration.

Performance may decrease if Dt is not a multiple of the simulation's time step (dt).

When CVode is active, only a Dt value of "-1" will prevent performance issues.

Simulation Controls:

Start simulation: Button to begin the simulation with the current parameters.

Save animation to BIN file: Checkbox to save the animation data in a binary format.

Save animation to TXT file: Checkbox to save the animation data in a text format.

Axon myelinisation (Neuron mode only)

To create a neuron with a myelinated axon, start by loading the neuron model. If the neuron already has an axon, you can directly proceed with myelination by importing the existing cell. If there is no axon present, you have the option to add an artificial axon or create one manually.

Once the neuron is ready, initiate the myelination process by selecting **Other Species**, followed by **Axon Simulation**.

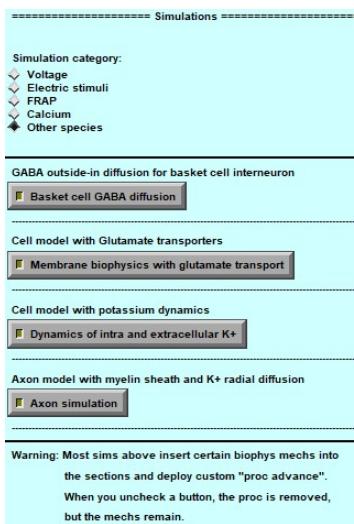


Figure 37. This panel provides options for simulating a neuron model with a myelinated axon. You can import an existing cell with an axon, create one from scratch, and then apply the myelination function.

After clicking "**Axon Modelling**" the main control panel (**Fig.38**) for creating and simulating an axon with myelin (Schwann cells) will appear. This panel is divided into several sections that allow you to configure various aspects of the axon model:

1. Geometry and Sheath

- Configure axon geometry: import, use predefined shapes, or create manually.
- Manage the myelin sheath: deploy, scalp, or remove.

2. Myelin Parameters

- Define regions of myelination.
- Set parameters for Schwann cells.

3. Axon Trunk Parameters

- Specify axon length, diameter, and segmentation.

4. Axon Sheath Parameters

- Define Schwann cell diameter and the number of radial shells.

5. Biophysics Parameters and Clamps

- Select between realistic biophysics or test sine wave simulations.
- Apply clamps to the soma.

6. Diffusion Parameters

- Set the diffusion coefficient and potassium concentrations.

7. Visualization Parameters

- Choose from various visualisation options to enhance simulation analysis.

The left side of the panel displays a visual representation of the axon model. Use the input fields, checkboxes, and sliders to adjust parameters as needed. Once configured, click "Start simulation" to run your model.

Note: Some parameters may affect others or require specific configurations. Refer to the detailed sections of this manual for more information on each parameter and its effects on the simulation.

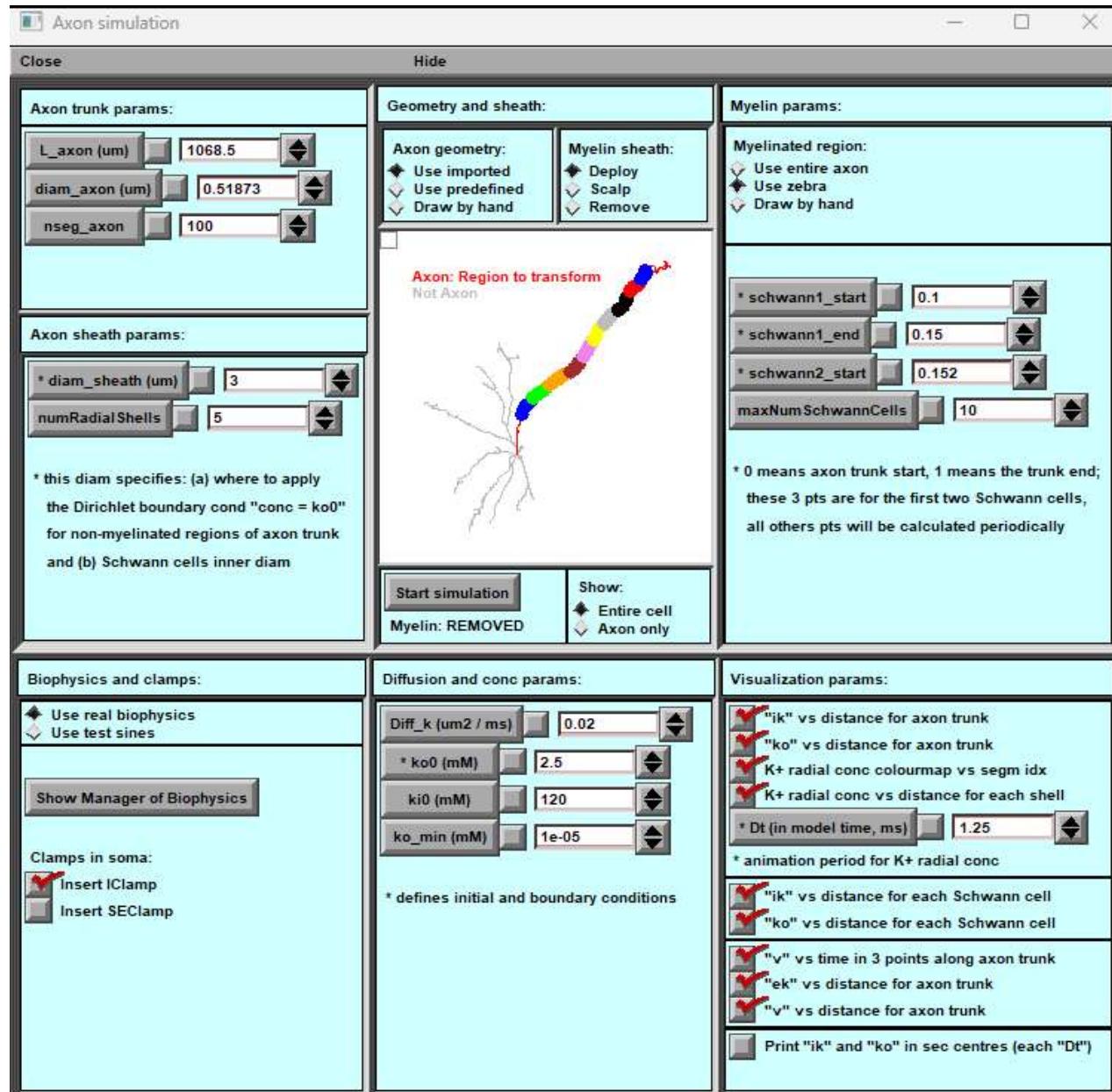


Figure 38. Axon Myelination Panel. **Geometry and Sheath:** Define axon geometry (import, predefined, or manual), deploy/remove myelin, and specify the region to transform into myelinated sections. **Myelin Params:** Choose regions for myelination, adjust Schwann cell properties (start, end positions), and set the maximum number of cells. **Axon/Sheath Params:** For sheath visualisation, configure axon length, diameter, segment count, and radial shells. **Biophysics and Clamps:** Add biophysical properties or use

predefined parameters and test waves. **Diffusion Params:** Define ion diffusion coefficients and concentrations for simulation. **Visualisation Params:** Select which simulation data (e.g., ion concentration or voltage) to visualise over time and space.

Geometry and Sheath Panel

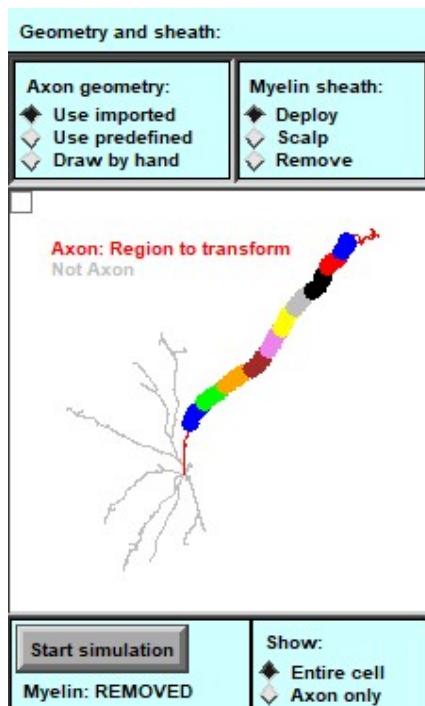


Figure 39. Axon Simulation Panel Overview. This panel is essential for designing and configuring a myelinated axon model. It contains two key sections:

1. Axon Geometry:

- **Use Imported:** Load the axon from a pre-existing cell model.
- **Use Predefined:** Apply the default axon structure from BrainCell.
- **Draw by Hand:** Manually sketch the axon.

2. Myelin Sheath:

- **Deploy:** Adds a myelin sheath to the axon, simulating its insulating properties.
- **Scalp:** Disables any milling mechanisms while preserving the shell geometry of the myelin sheath.
- **Remove:** Completely remove the myelin sheath, leaving a uniform axon for simulation.

Visual representations highlight the axon, showing different segments. The "Start Simulation" button initiates the simulation, and a status indicator reflects the myelination state (e.g., "Myelin: REMOVED").

Note: Changes here affect other settings; review related panels to ensure consistency.

Draw Axon Panel Overview

When you select "Draw by hand" in the **Geometry and Sheath** panel, NEURON's **CellBuilder** window opens, allowing for manual construction of neuronal structures, including axons.

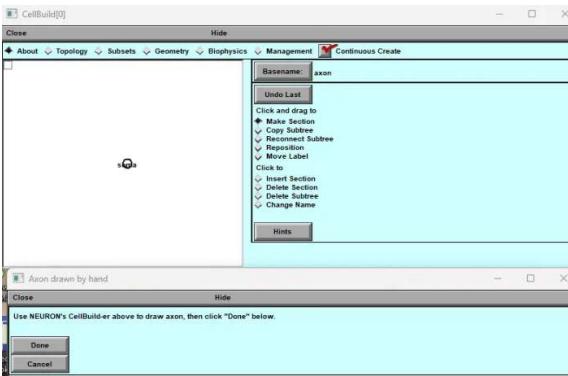


Figure 40. Drawing Canvas (Left):

- The white space where the axon is visually constructed.
- Initially displays a circular point, representing the axon's start.

Control Panel (Right):

- Offers options like **Make Section**, **Undo Last**, and **Reconnect Subtree** for building and editing the axon.
- Buttons for inserting, deleting, and renaming axon segments.

Hints button for additional instructions.

Completion: Below the window, the "**Axon drawn by hand**" panel provides options:

Done: Saves and exits. **Cancel**: Discards changes.

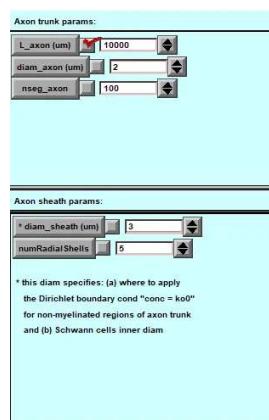
Usage Tips: Construct the axon using the available drawing tools.

Use **Undo Last** for corrections. Save the structure with **Done** when complete.

Note: It may take practice to master the **CellBuilder** interface, so take your time experimenting with its features.

Axon Parameters Panel Overview

This panel is used to configure the axon's physical properties and its myelin sheath. It consists of two main sections:



1. Axon Trunk Parameters:

- **L_axon (μm)**: Defines the axon length in micrometres (default: 10,000 μm).

- **diam_axon (μm)**: Specifies the axon diameter (default: 2 μm).
- **nseg_axon**: Number of segments modelling the axon (default: 100).

2. Axon Sheath Parameters:

- **diam_sheath (μm)**: Diameter of the myelin sheath (default: 3 μm).
- **numRadialShells**: Number of concentric shells modelling the sheath (default: 5).

Important: The **diam_sheath** also applies boundary conditions to non-myelinated axon regions and sets the inner Schwann cell diameter.

Adjust parameters with caution, as they significantly impact the model's accuracy.

Myelin Parameters Panel

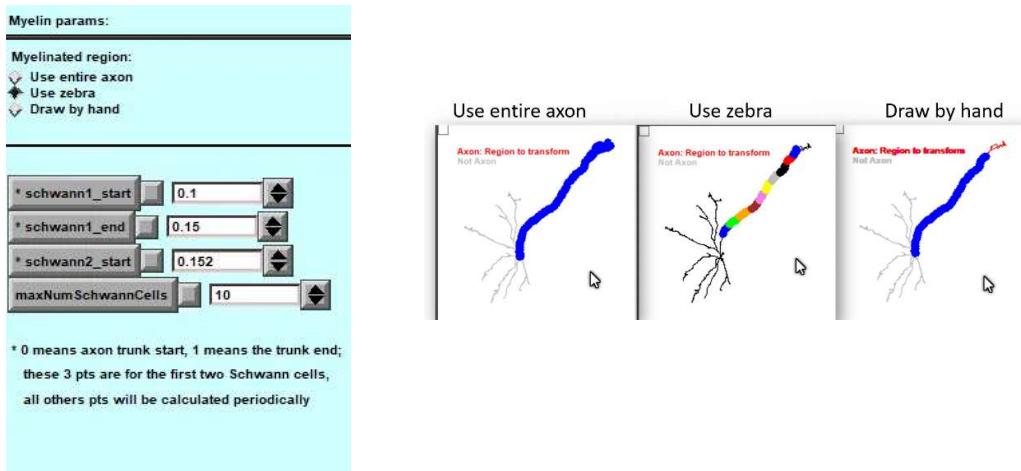


Figure 41. This panel lets you configure the properties of the myelin sheath. It consists of two key sections:

1. Myelinated Region Selection:

- **Use entire axon**: Myelin covers the entire axon.
- **Use zebra**: Alternating myelinated and unmyelinated sections along the axon.
- **Draw by hand**: Manually define the myelinated regions.

2. Schwann Cell Parameters:

- **schwann1_start**: Defines where the first Schwann cell starts (e.g., 0.1).
- **schwann1_end**: Defines the end of the first Schwann cell (e.g., 0.15).
- **schwann2_start**: Starting position of the second Schwann cell (e.g., 0.152).
- **maxNumSchwannCells**: Maximum number of Schwann cells (e.g., 10).

Positions are relative (0 = start, 1 = end of axon trunk). Visual guides in the panel provide feedback for your selections.

Each option shows a different myelination pattern:

- **Entire axon**: Fully myelinated (shown in blue).
- **Zebra**: Alternating myelin and non-myelinated regions (various colors).
- **Draw by hand**: Custom regions.

Diffusion and Concentration Parameters Panel

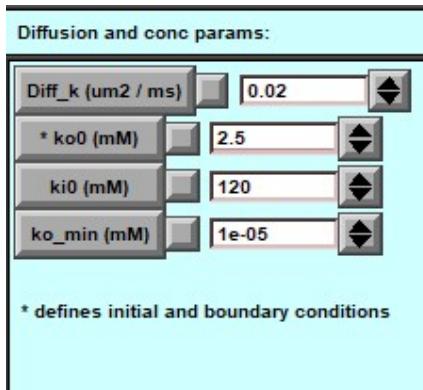


Figure 42. This panel lets to set potassium diffusion and concentration values for myelinated axon simulation.

1. **Diff_k (um²/ms):** Sets the potassium ion diffusion coefficient (e.g., 0.02), controlling how quickly potassium spreads.
2. **ko0 (mM):** The initial extracellular potassium concentration (e.g., 2.5), also used as the boundary condition.
3. **ki0 (mM):** Initial intracellular potassium concentration (e.g., 120 mM).
4. **ko_min (mM):** The minimum extracellular potassium concentration (e.g., 1e-05 mM) to prevent potassium depletion.

Carefully adjust these values to model physiological conditions, as they affect ion movement and action potential propagation during the simulation.

Biophysics and Clamps Panel

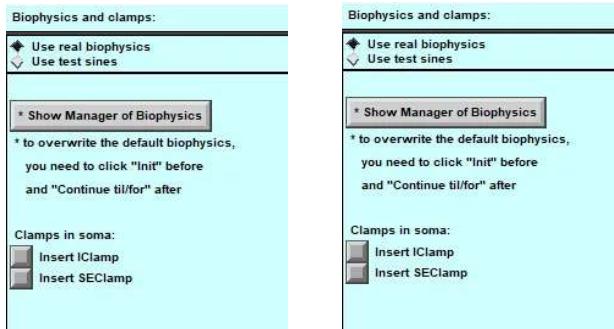


Figure 43. This panel allows you to configure the biophysical properties and experimental manipulations for your axon simulation. The panel has two different configurations, which I'll describe below:

- Use real biophysics: Selected
- Use test sines: Not selected

Configuration 1: Realistic Biophysics

- **Use real biophysics:** Activates realistic models for axonal behaviour.
- **Show Manager of Biophysics:** Opens an advanced settings panel.
- **Clamps:** Options to insert current (IClamp) or voltage clamps (SEClamp) into the soma.
 - *Tip:* Click "Init" before and "Continue til/for" after adjusting overwrite defaults.

Configuration 2: Test Sine Waves

- Use real biophysics: Not selected
 - Use test sines: Selected
 - **Use test sines:** Uses simplified sine waves for controlled testing.
 - **Clamps:** Same options available for experimental manipulation.
-

User Notes:

- Use real biophysics for accurate physiological simulations and test sines for simplified experiments.

Visualisation of computation

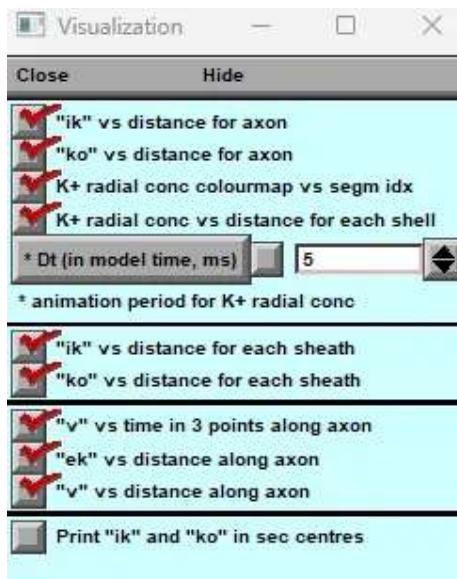


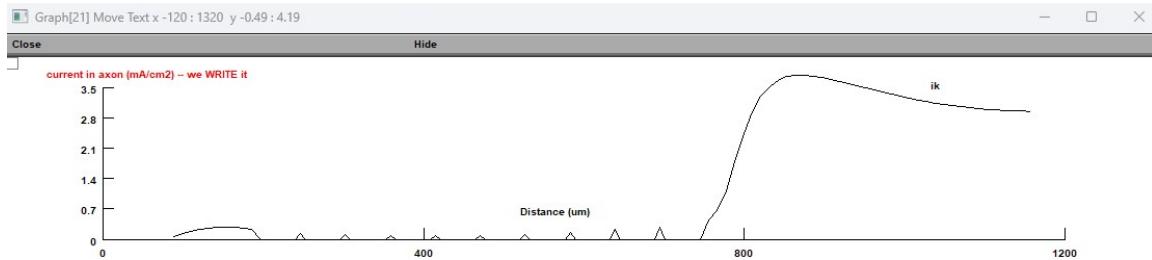
Figure 44. Panel for visual results.

- Checkbox (checked): Indicates that the option is enabled or selected
- Checkbox (unchecked): Indicates that the option is disabled or not selected
- ▼ - Dropdown arrow: Allows adjusting numerical values

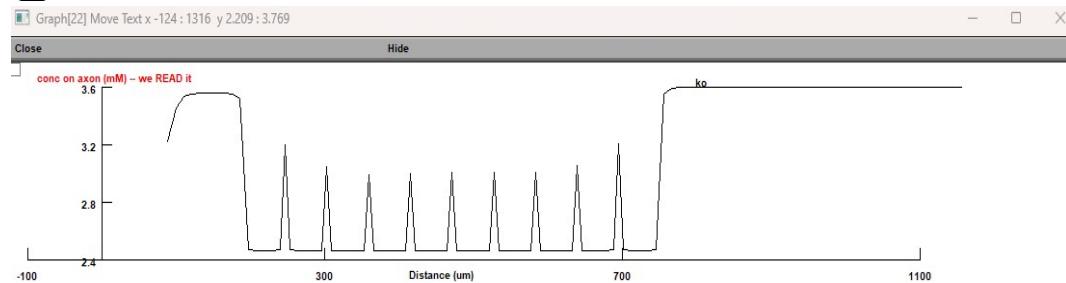
Legend for visualisation options:

1. "ik" vs distance for axon

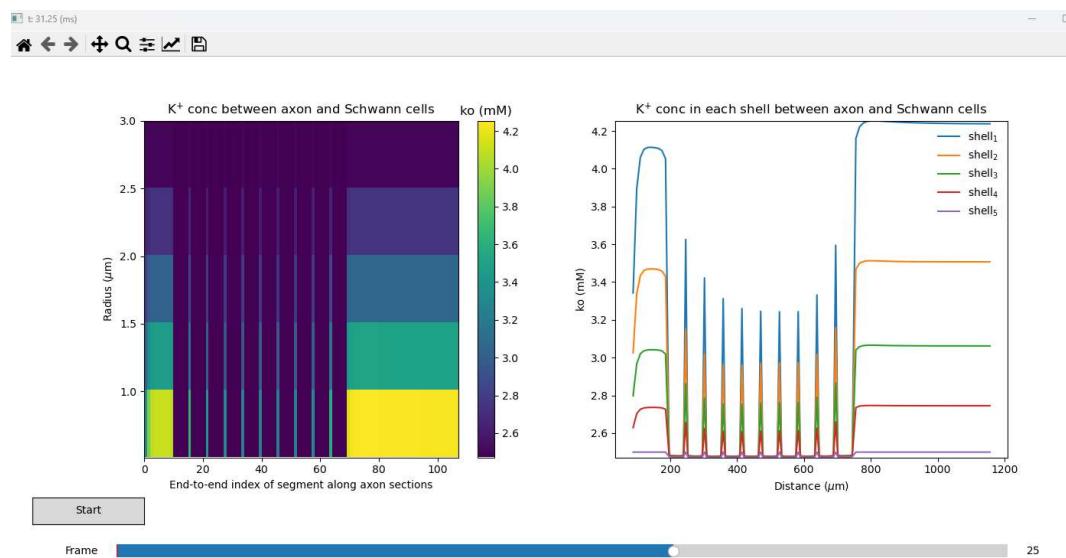
Here is an example of plots if the user keeps these options.



2. "ko" vs distance for axon

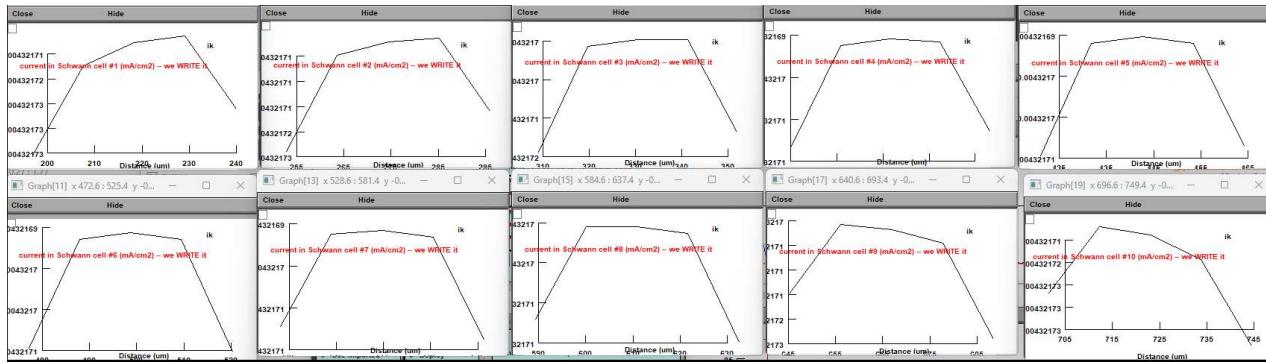


3. [K]⁺ radial concentration colourmap vs segment idx
4. [K]⁺ radial concentration vs distance for each shell
5. Here is an example of plots if the user keeps these two options.



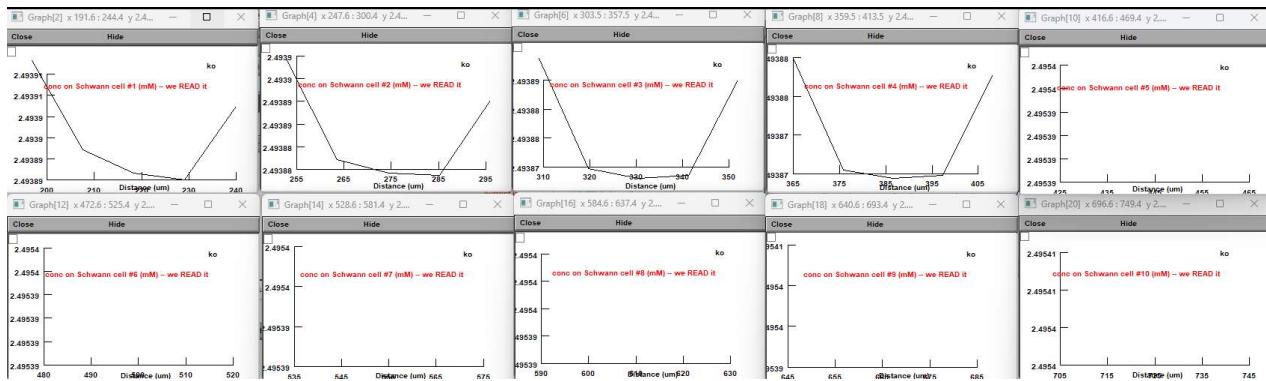
6. Dt (in model time, ms): 20 [Numerical input field with dropdown] * animation period for K⁺ radial concentration
7. "ik" density of potassium current vs distance for each myelinated sheath

Here is an example of plots if the user keeps these options.



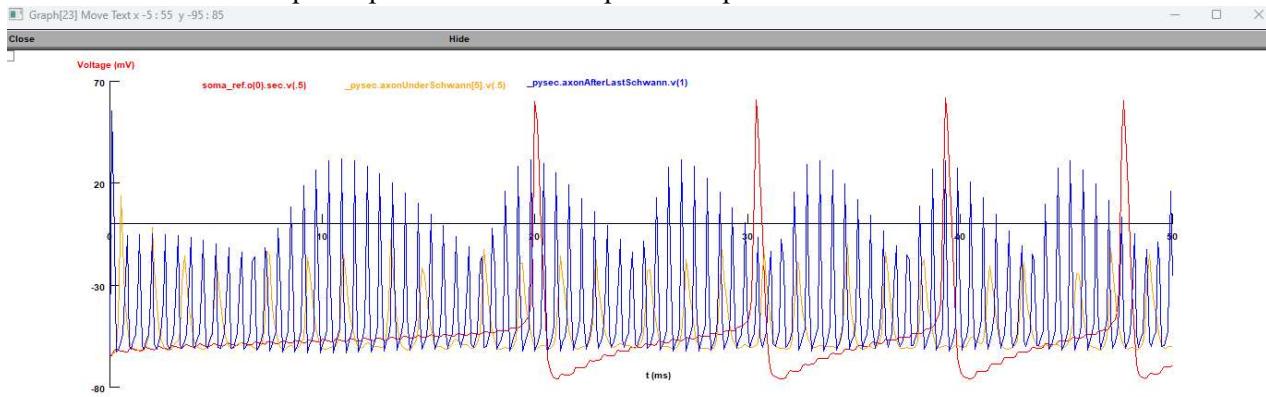
8. "ko" [K]o extracellular potassium concentration vs distance for each myelinated sheath

Here is an example of plots if the user keeps these options.

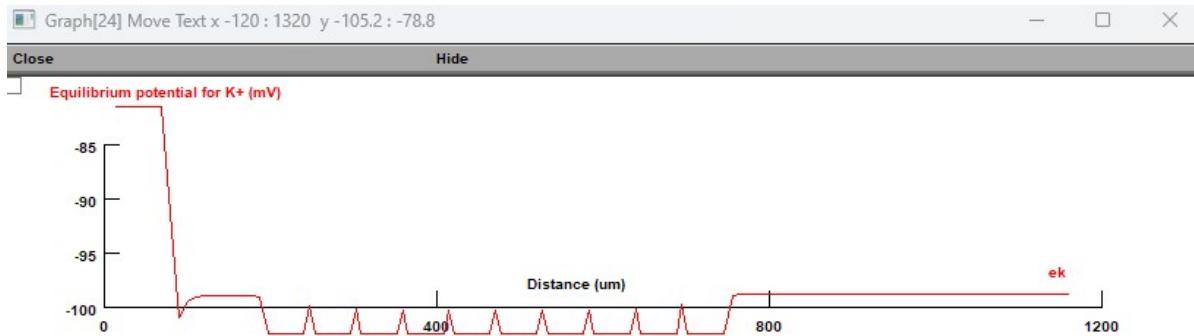


9. "v" axon membrane voltage vs time in 3 points along axon trunk

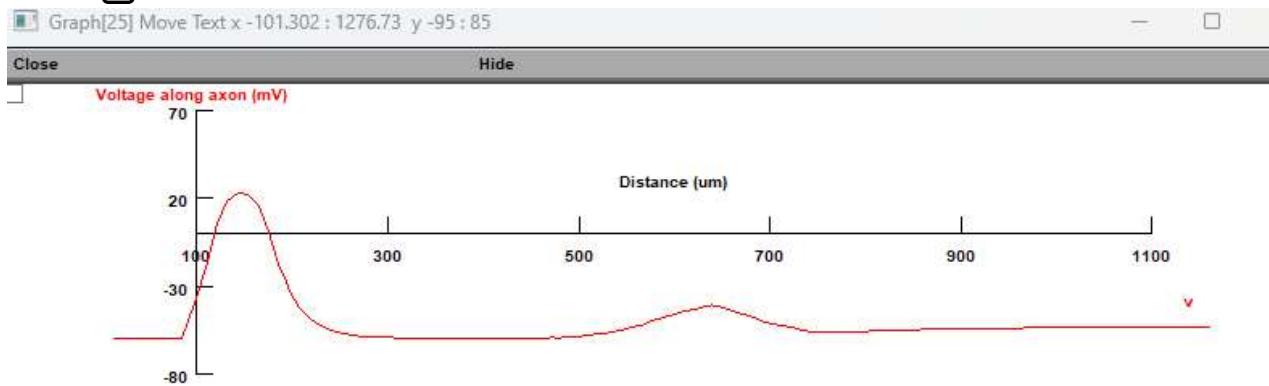
Here is an example of plots if the user keeps these options.



10. "ek" potassium reverse potential on axon branches vs distance for axon trunk



11. "v" vs distance for axon trunk



11. Print "ik" and "ko" in sec centres [Unchecked option]

LFP. Recording local field potential for a single cell.

Local field potentials (LFPs) represent the aggregate electrical activity of neuronal populations and are a key tool for studying the interactions between neural circuits. The LFP simulation module in BrainCell allows researchers to calculate extracellular potentials at specific locations around a neural structure based on user-defined parameters. This tool is particularly useful for investigating how neural activity propagates through space and interacts with surrounding tissues.

The interface is designed to be intuitive and flexible. It offers customisation for electrode placement, simulation parameters, and stochastic modelling to accommodate diverse experimental needs. Users can visualise real-time results, ensuring an interactive and efficient workflow.

This interface (**Figure 45**) allows users to configure and run simulations of local field potentials (LFPs) in neural structures, providing real-time feedback and visualisation.

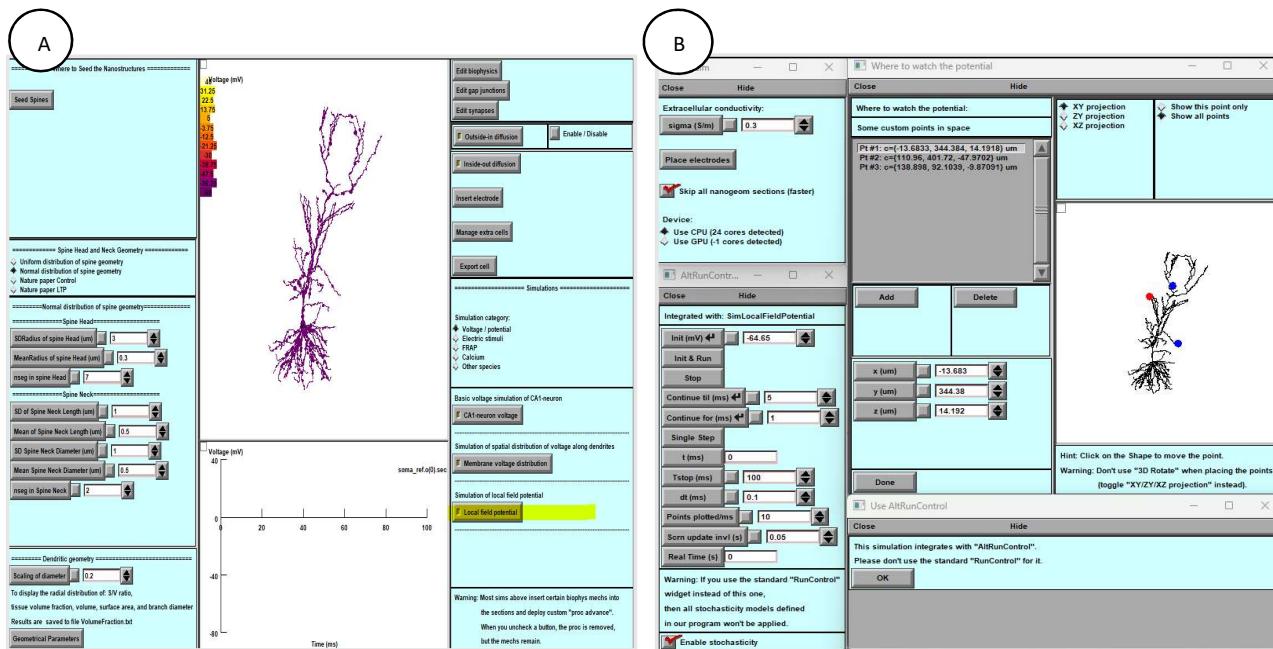


Figure 45. Local Field Potential (LFP) Simulation Panel. (A) **Main panel.** Activating Local Field Potential (LFP) Simulation. To enable LFP simulation, use the Simulations menu on the right. (B) This panel allows users to configure and run simulations of local field potentials (LFPs) in neural structures, providing real-time feedback and visualisation.

Sub panels.

1. **Extracellular Conductivity (σ):**
2. Adjust the conductivity of the extracellular medium in Siemens per meter (S/m).
 - o Default value: 0.3 S/m.
 - o Modify based on the experimental conditions or tissue type.
3. **Electrode Placement Options:**
 - o **Place Electrodes:** Opens a sub-panel to define custom points in space for potential monitoring.
 - o **Skip All Nanogeom Sections:** Ignoring small geometry sections improves simulation speed.

4. **Device Selection:**
 - Choose between CPU and GPU for simulation execution. The panel indicates the number of available cores for each.
 5. **"Where to Watch the Potential" Sub-panel:**
 - **Custom Points in Space:** List of coordinates (in micrometres) where potentials will be calculated.
 - **Visualization Tools:**
 - Toggle between XY, XZ, and YZ projections of the neural structure.
 - Points are shown as coloured dots on the neural structure for reference.
 - Use the "Add" and "Delete" buttons to manage observation points.
 6. **Alternative Run Control (AltRunControl):**
 7. This customised simulation control panel integrates stochastic modelling features. **Do not use standard RunControl when AltRunControl is active.**
- Key Parameters:**
- **Initialization (Init):** Set the initial voltage (in mV).
 - **Run Control:**
 - Define simulation start and stop times (ms).
 - Configure plotting intervals and points displayed per millisecond.
 - **Enable Stochasticity:** Check this box to activate stochastic models in the simulation.
- Real-Time Monitoring:**
- Specify the screen update interval (in seconds) to adjust visualisation refresh rates during real-time simulations.

Visualisation and Controls:

- **3D Shape Viewer:** Interact with the neural structure to adjust point positions visually. Avoid using "3D Rotate" mode for precise positioning.

Warnings:

- When using AltRunControl, standard RunControl widgets are disabled to ensure compatibility with stochastic models.
-

Submitting BrainCell Export Files for Simulation on NSG Neuroscience Gateway

8. Archive Preparation

Ensure the archive contains a single folder with all required files inside. Choose a simple folder name without Unicode characters, spaces, or excessive length.

Open the main HOC file (e.g., "runner.hoc") and prepend with { load_file("stdrun.hoc") } for Linux compatibility. This ensures NEURON recognizes standard procedures.

Specify the main input file correctly in task parameters, typically "runner.hoc". Note: Cloning tasks may reset this field.

9. Necessary Files

Linux systems require MOD files instead of "nrnmech.dll". Combine MOD files from "Common" and either "Astrocyte" or "Neuron" folders into the same folder as your HOC file. MOD files will compile automatically into "libnrnmech.so".

10. Parameter Settings:

Set the following parameters to 1, as BrainCell does not yet support parallel computing:

Number of Nodes

MPI Tasks per Node

OpenMP Threads per Task

Cores per Node Reducing requested resources may decrease queue time.

11. Additional Observations:

"NEURON on Expanse" is sufficient for running BrainCell files that require NEURON and Python.

Submission to computation time varies; it can range from 30 minutes to 15 hours.

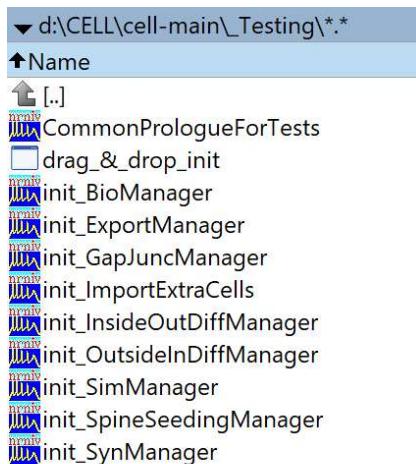
Errors may occur with "Core NEURON on Expanse", and "NSG submissions are temporarily halted on Expanse" can prevent task submission.

This manual is designed to guide users through preparing and submitting BrainCell export files for simulation on the NSG platform, highlighting key steps and tips for successful computation.

BrainCell Testing Suite

The BrainCell software provides a comprehensive set of testing tools located in the directory:
...\\cell-main\\Testing\\

This suite allows you to test and initialise various components of the BrainCell simulation environment.
Below is an overview of the available testing modules:



This suite allows you to test and initialise various components of the BrainCell simulation environment.
Below is an overview of the available testing modules and how to use them:

1. CommonPrologueForTests

- Purpose: An internal utility file used by other tests.

Note: This is not an entry point for users. Other test scripts automatically call it as needed.

2. drag_&_drop_init.bat

- Purpose: A utility script to simplify running tests.

- Usage: In Windows Explorer, drag any "init_*\\.hoc" test file and drop it onto "drag_&_drop_init.bat" to execute the test.

3. init_BioManager

- Purpose: Initializes and tests the Biological Properties Manager.

4. init_ExportManager

- Purpose: Sets up and tests the Export functionality manager.

5. init_GapJuncManager

- Purpose: Initializes and tests the Gap Junction Manager.

6. init_ImportExtraCells

- Purpose: Test the system for importing additional cell models.

7. init_InsideOutDiffManager

- Purpose: Initializes and tests the Inside-Out Diffusion Manager.

8. init_OutsideInDiffManager

- Purpose: Sets up and tests the Outside-In Diffusion Manager.

9. init_SimManager

- Purpose: Initializes and tests the main Simulation Manager.

10. init_SpineSeedingManager

- Purpose: Test the Spine Seeding Manager.

11. init_SynManager

- Purpose: Initializes and tests the Synapse Manager.

To run these tests:

Method 1: Using drag_&_drop_init.bat

1. Open Windows Explorer and navigate to ...\\cell-main_Testing\\
2. Locate the "drag_&_drop_init.bat" file.
3. Find the "init_*.hoc" file for the component you want to test.
4. Drag the chosen "init_*.hoc" file and drop it onto "drag_&_drop_init.bat".
5. The test will automatically run.

Method 2: Direct Execution

1. Navigate to the ...\\cell-main_Testing\\ directory.
2. Double-click on the appropriate "init_*.hoc" file to run the specific test.

After running a test:

- Review any output or logs generated by the test to verify the component's functionality.
- Check for any error messages or unexpected behaviour.

Note: Each test script is designed to run independently and will call the CommonPrologueForTests internally if needed. There's no need to run CommonPrologueForTests separately.

Please refer to the individual documentation files accompanying each init script for detailed information on each testing module and specific testing procedures.

Creating and Editing Biophysical Mechanisms

BrainCell allows you to create and modify sets of biophysical mechanisms for cell modeling. While you can use the built-in Biophysical Mechanisms software, you also have the option to create and edit these mechanisms directly using JSON files. This method provides a simple and visual approach using any text editor.

Location of JSON Files

The JSON files for biophysical mechanisms are located in the following directory:

...\\cell-main\\Biophysics\\



This directory contains three subdirectories:

1. [Neuron]: Mechanisms specific to neurons
2. [Astrocyte]: Mechanisms specific to astrocytes
3. [Common]: Mechanisms shared between different cell types

Creating a New JSON File

1. Navigate to the appropriate subdirectory ([Neuron], [Astrocyte], or [Common]).
2. Create a new text file with a descriptive name (e.g., "MyNewMechanism.json").
3. Open the file in a text editor of your choice.

Editing JSON Files

1. Open the desired JSON file in a text editor.
2. Follow the JSON syntax to define your biophysical mechanisms.
3. Each mechanism should be defined with its properties and parameters.

Example JSON Structure:

```
{  
  "MechanismName": {  
    "type": "voltage_gated_channel",  
    "ion": "na",  
    "equations": {  
      "alpha_m": "0.1 * (v + 40) / (1 - exp(-(v + 40) / 10))",  
      "beta_m": "4 * exp(-(v + 65) / 18)"  
    },  
    "parameters": {  
      "V": -70,  
      "E_Na": 55,  
      "E_K": -90  
    }  
  }  
}
```

```
        "gmax": 120,  
        "e_rev": 50  
    }  
}  
}
```

Available Mechanisms

As shown in Image 2, some of the available mechanisms for neurons include:

- AllMyelin
- Myelin
- SimCalcium
- SimFrap
- SimGlutamate
- SimMyelinatedAxon
- SimPotassium
- SimVoltageCA1Neuron

You can create new mechanisms or modify existing ones based on these templates.

Best Practices

1. Use clear, descriptive names for your JSON files and mechanisms.
2. Comment your JSON files using // for single-line comments or /* */ for multi-line comments to explain complex parts.
3. Validate your JSON syntax using online tools before saving to ensure it's correctly formatted.
4. Back up original files before making significant changes.

Applying Changes

After editing or creating a JSON file:

1. Save the file in the appropriate subdirectory.
2. Restart the BrainCell software or reload the biophysical mechanisms to apply the changes.

Note: Always ensure that your modifications are scientifically accurate and consistent with the overall model you're developing.

This method allows you to easily create, view, and edit complex biophysical mechanisms in a familiar text-based format, providing flexibility in your modelling approach.