**User Manual**

**SyncroPatch automated data analysis for NaV1.5**

Accompanying script: **NaV\_clean.m**

Accompanying paper: **Multi-site validation of a functional assay to adjudicate *SCN5A* Brugada Syndrome-associated variants**

Supplemental reading: Ma JG, Vandenberg JI and Ng C-A (2023), Development of automated patch clamp assays to overcome the burden of variants of uncertain significance in inheritable arrhythmia syndromes. Front. Physiol. 14:1294741. doi: 10.3389/fphys.2023.1294741

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# **Overview**

This user manual outlines how to use the MATLAB script for the automated analysis of electrophysiological datasets obtained from the SyncroPatch 384 PE (Nanion Technologies). This script was designed to analyse current densities, steady-state activation, steady-state inactivation and recovery from inactivation using protocols as specified.

There are also notes throughout the script to specify variables that may be changed and for guidance.

# **Project requirements**

The programming language used in this analysis is MATLAB (MathWorks; <https://au.mathworks.com/products/matlab.html>).

This project was designed and ran using MATLAB version 2022a.

# **Analysis pipeline**

## **Prior to exporting: Experimental set up**

### Experimental layout

This script will obtain cell and variant ID from the DataControl exports. Therefore it is important that during experimentation these data are inputted correctly in PatchControl (but may also be added/editted later in DataControl). Specifically, under the cell layout (green) the variant ID should be labelled per column as required. See figure below where cells were separated into 12 columns by variant and the corresponding variant ID is indicated by a value with the corresponding variant ID.

A screenshot of a computer

Description automatically generated

### Voltage protocols

The experimental methods and specific voltage protocol used in this experiment are described in the accompanying research paper. See figure below for outline of voltage protocol.

A graph of a number of red and black lines

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A graph with numbers and lines

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A graph with numbers and lines

Description automatically generated

A graph with numbers and a line

Description automatically generated with medium confidence

A graph with numbers and lines

Description automatically generated

## **Data export from DataControl**

Data is exported from DataControl384 (Nanion Technologies). PatchClamp contains inbuilt automated QC capabilities (set to parameters as required – e.g. Rseal > 500 MO, capacitance = 2-30 pF, Rseries < 50 MO). See figure for voltage protocol used. In summary, a leak

### Exporting raw traces

Under the QC selection tab, export trace data (all sweeps) using the settings: export entire chip, export all sweeps, export leak corrected data, use column headers. Only ignore QC filter if you wish you export all cells including those that did not pass the automated QC. Export using Tab as separator and Format undefined as NaN. See figure below (left) for exporting traces and (right) for configuration of names used in this project.

A screenshot of a computer

Description automatically generated

### Exporting QC

Under the Results tab, export QC data including Well ID, Cell Type (variant ID), Sweep result block (seal resistance, capacitance, series resistance). See figure below.

A screenshot of a computer

Description automatically generated

## **Running the script (CD/SSA/SSI)**

This script was written and ran using MATLAB (2022). General variables (QC parameters) have been placed at the top of the script for easy modifications.

### Data import into MatLab

The script has the details of the import written in so if previous export were as followed, there are no additional requirements.

### Input variables

* 1. SSA times, SSI times, leak times

This indicates the start and end points for analysis (cursors) of SSA, SSI and leak currents.

* + 1. SSA measures the activation (peaks) of the channels occurring when the voltage depolarises. i.e. activation at 350ms. The SSA end cursor is set to 10ms after depolarisation and therefore all peaks are measured during this time. SSA end time may be set earlier if desired as NaV typically take approx. 1ms to activate.

See voltage protocol above.

* + 1. SSI measures the inactivation (peak tails) of the channels occurring after the voltage have depolarised for a set time (500ms). For NaV.. i.e. inactivation is measured at 850ms for 3ms. Refer to voltage protocol above.
    2. Leak assists in eliminating leaky low quality cells. In the protocol above, there is a small repolarising step to -120mV for 50ms. The conductance at this brief voltage step is determined to be leaky if it exceeds ±40pA. (This step may also be inputted directly into PatchControl to automatically QC alongside Rseal, Cslow, Rseries).
  1. Voltages
     1. Voltages used are inputted as a range. Do not list all voltages.
     2. For sweep voltages starting from -120mV and increasing at 5mV increments to +60 mV, the input are as follows:

[initial voltage : interval : end voltage ] = [-120:5:60]

* 1. Time to peak

This variable is set to evaluate the time at which the peak conductance occurs at 0mV. Lower and upper limits determine the thresholds acceptable for the “fastest” and “slowest” time desired. For the analysis of sodium currents, the lower and upper peaks were set to 0.5ms and 1.1ms, respectively.

* + 1. The units used are microseconds (us). i.e. 1.1ms = 1100 us
  1. Conductance thresholds

This QC variable is applied during the evaluation of SSA and SSI. For NaV, under experimental conditions described in manuscript above, conductance greater than 2 nA were associated with greater likelihood of voltage clamp errors and poor cell quality whist small conductance do not enable an accurate calculation of cell activation and inactivation voltages.

* + 1. The units used are in amperes (A). i.e. 200 pA = -0.0000000002
  1. Directories

In the project script, names are indicated by quotation marks and the purple colour. E.g. ‘Raw\_SSA’.

Directories have been split into parent-, experimental-, protocol- directories for ease. Simply set up the parent directory once and change experimental/protocol directory as required for each experimental date/chip to be analysed. Throughout the script, full directories will be joined to summon specific locations.

* + 1. Outline of directories example

### Automated analysis

All automated analysis will be saved into an auto-generated folder called ’PostQC’.

* 1. PeaksAndTimes

The peaks are identified by finding the mins/maxs and the time they occur. The peaks are used for the calculation at later steps. The time is used for the QC, time to peak. The output of this step also includes folders SSA and SSI containing the plots for activation traces per sweep, activation by voltage, inactivation traces per sweep and inactivation by voltage. Another output is a file containing the calculated reversal potentials. The reversal potential is calculated using a linear best fit through peaks from 0mV to +60mV and extrapolating the x where y=0.

* 1. CurrentDensity

Current Density is calculated using the formula peak/cslow at each sweep, saved as ‘CD\_persweep’. The CD summarised (means) for each variant in that experimental chip is saved as ‘CD\_summary’. The sweep of interest (-20mV) is further used for cell analysis – CD, square-root CD, CD normalise to WT(mean), and sqrtCD normalised to sqrtWT(mean).

* 1. SteadyStateActivations\_V50

SSA V50 is calculated per cell using the Boltzmann function and the previously calculated reversal potential. Outputs, for each cell that had passed QC, includes the calculated V50, each V50 minus the WT mean V50, each slope and R2 for the specific curve, and the normalised peaks used to calculate the Boltzmann function. Data is also summarised for each variant in a separate excel file.

* 1. SteadyStateActivations\_plots

The plots of cells with SSA calculated.

* 1. SteadyStateInactivations\_V50

SSI V50 is calculated per cell using the Boltzmann function. Outputs, for each cell that had passed QC, includes the calculated V50, each V50 minus the WT mean V50, each slope and R2 for the specific curve, and the normalised peaks used to calculate the Boltzmann function. Data is also summarised for each variant in a separate excel file.

* 1. SteadyStateInactivations\_plots

SSI plots per cell.

### Project outputs

There are two types of outputs: excel files containing data and plots in the form of jpg (but can be modified to pdf or format of your choosing). See flowchart below for specific csv files and the locations they will be exported to.

### Output: data

### Outputs: figures

A graph showing a number of different colored lines

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A graph of a graph with a line

Description automatically generated with medium confidence

A graph of a voltage

Description automatically generated A graph of a voltage

Description automatically generated

## **Running the script (RFI)**

The analysis for RFI is written separately as it involved a separate voltage protocol that has been split into 4 protocols due to time and step constraints (per protocol) on the Syncropatch. Therefore this protocol amalgamates the 4 RFI protocols for analysis.

### Data import into MatLab

The script has the details of the import written in so if previous export were as followed, there are no additional requirements.

### Input variables

* 1. RFI times, leak times

This indicates the start and end points for analysis (cursors) of SSA, SSI and leak currents.

* + 1. RFI calculates the recovery from inactivation following different time intervals. In this project the time intervals are 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 200, 500 and 1000 ms. These times have been split across 4 protocols due to protocol constraints on the SyncroPatch. Time cursors in the project reflect the depolarisations to -20mV occurring at the test-pulse and is normalised to a control pulse (see voltage protocols above).
    2. Leak assists in eliminating leaky low quality cells. In the protocol above, there is a small repolarising step to -120mV for 50ms. The conductance at this brief voltage step is determined to be leaky if it exceeds ±40pA. (This step may also be inputted directly into PatchControl to automatically QC alongside Rseal, Cslow, Rseries).

### Automated analysis

All automated analysis will be saved into an auto-generated folder called ’Recoveries’ under the first recovery protocol folder.

* 1. Recoveries

RFI calculates the recovery from inactivation by measuring the peak at each voltage depolarisation occurring at the test-pulse (which follows a pre-pulse with increasing time intervals between the two pulses to determine the percentage recovery at each time interval). All peaks measured are normalised to a control peak that occurs at the beginning of the protocols. All data is normalised between 0 and 1 for evaluation.

Data is then plotted first using a double exponential equation to obtain the time each cell takes to reach a half-recovered state, followed by a single exponential equation. Outputs recoded include the half time, half time of cell subtracting the WT mean half time, Kfast and Kslow, tau fast and tau slow, and R2 values, for each exponential analysis.

### Project outputs

There are two types of outputs: excel files containing data and plots in the form of jpg (but can be modified to pdf or format of your choosing). See flowchart below for specific csv files and the locations they will be exported to.

### Output: data

### Outputs: figures

