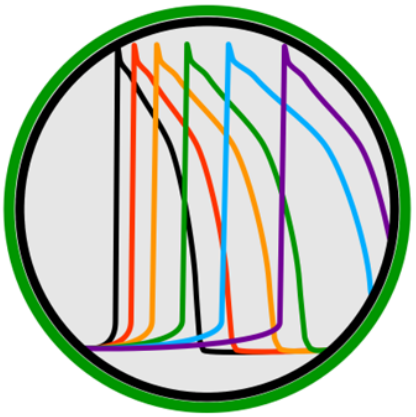


Action Potential Batch Analyser v.XXX

This script allows the automated analysis of triggered and spontaneous cardiac action potentials (APs) from adult, neonatal and hiPSC-derived cardiomyocytes recorded with the patch clamp technique.

IMPORTANT: Current version of BAPTA works with Axon Binary Files (***.abf***)

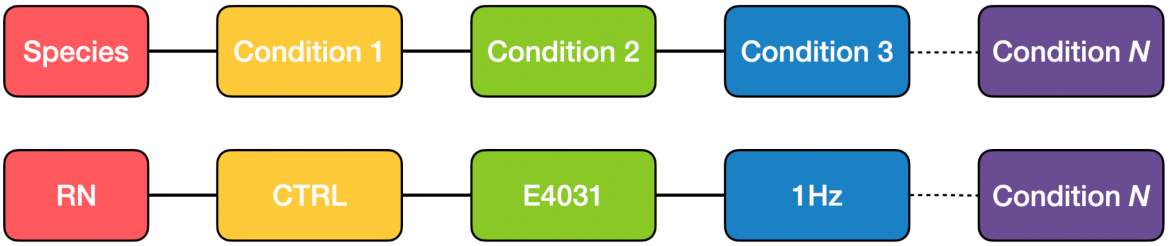
Installation

- ☐ **Web browser:** If you do not have a web browser please download and install a modern one, Google Chrome for instance: https://www.google.com/intl/ru/chrome/
  This step is important only if you need user interface, otherwise you can work directly in the interface of R-Studio
- ☐ **R:** Download and install R following the instructions for your specific operating system: https://cloud.r-project.org/
- ☐ **R-Studio:** Download and install R-Studio following the instructions for your specific operating system: https://www.rstudio.com/products/rstudio/download/#download
- ☐ **BAPTA:** You can clone a repository via GIT: https://github.com/l-sala/BAPTA.git
  or download zip archive from the same page: Press “Code” button, download ZIP, unzip archive to suitable directory.

Folder Structure Preparation

In the root directory of the script find the ***data*** folder (if not present, create it).
In the *data* folder import folders with ABF files. Naming of folders with files should follow template:

Folder names: Species_Group_Condition1_Condition2_...Condition`N`



It is mandatory to create one folder for each pacing frequency and/or for each condition. For example, if you have to analyse APs from Guinea Pig (Species) CMs, in control and in the presence of a drug (Group) at two different pacing frequencies or drug concentrations (Condition), we recommend to create 4 folders with the following structure:

- GP_CTR_PacingFrequency1
- GP_DRUG_PacingFrequency1
- GP_CTR_PacingFrequency2
- GP_DRUG_PacingFrequency2

or:

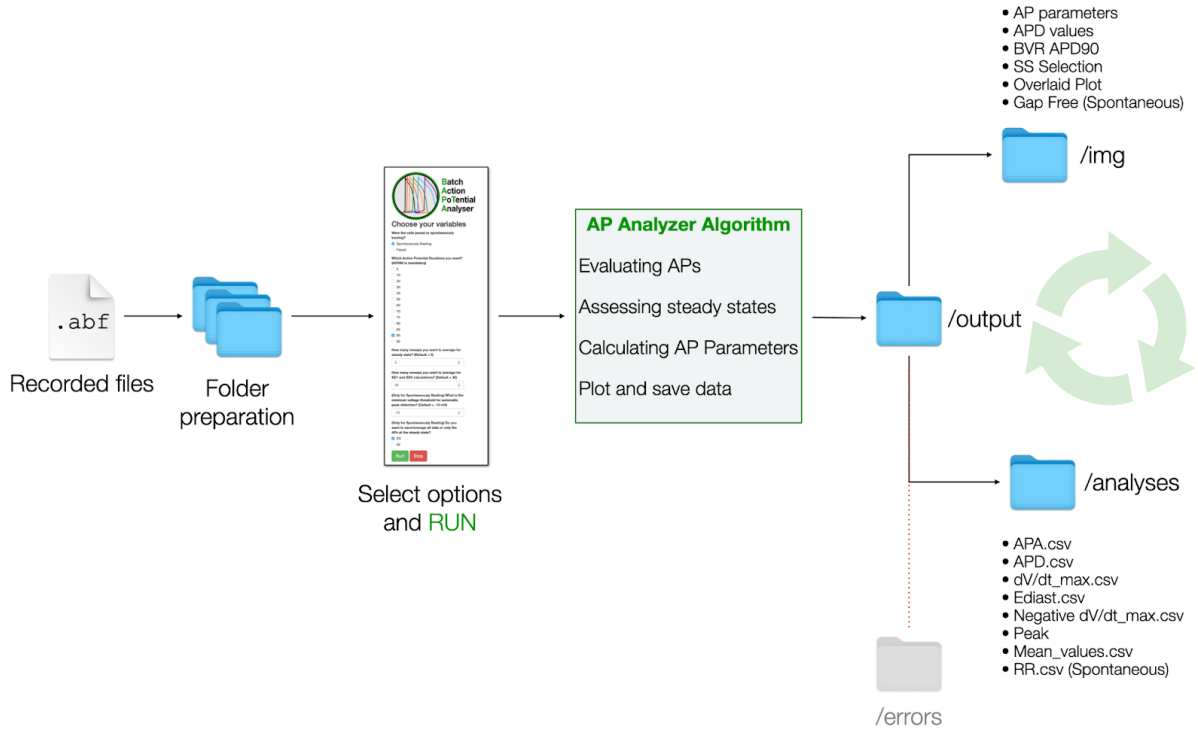
- hiPSC.CMs_Vehicle
- hiPSC.CMs_Isoprenaline_1μM
- hiPSC.CMs_Isoprenaline_10μM

IMPORTANT: Before start of the analysis it is strongly recommended to properly divide the .abf files in the proper folders.

- Any alteration in the *data* folder is prohibited during analysis.
- Prohibited to import both Paced and Spontaneous files at once. Analyze them separately!

How it works

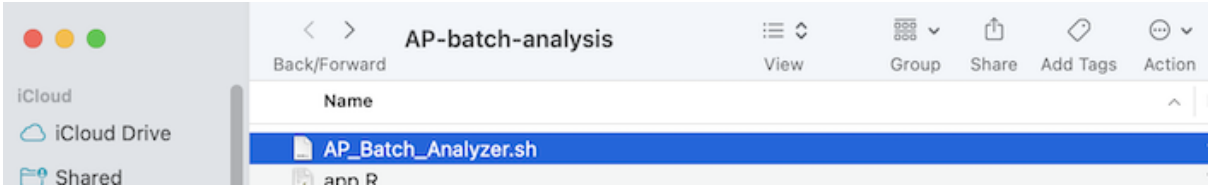
The tool identifies, within the time course of an AP file, the APs which have the lowest and most stable diastolic intervals. Among these, the tool generates groups of N (this number is customizable) subsequent APs. Among these groups, it will select the one characterised by lowest absolute APD_90 cumulative difference, and it will calculate and average all the parameters from these N APs. Default values for N are 5 for the calculation of AP parameters and 30 for STV calculations. With the exclusion of SD1 and SD2, each parameter will be calculated and described by a binary system of coordinates.



How to launch

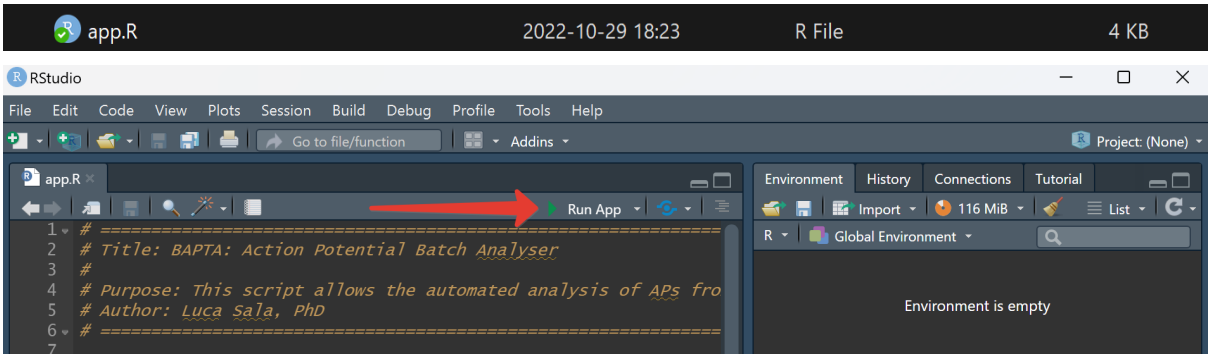
macOS and Linux

Launch by double-clicking **“AP_Batch_Analyzer.sh”** in the root directory. This will open a Terminal window and load the Shiny app interface.



Windows

Launch by double-clicking **“app.R”** in the root directory. This will load the Shiny app in RStudio; In the top side of the RStudio window, click ▶️ Run App.

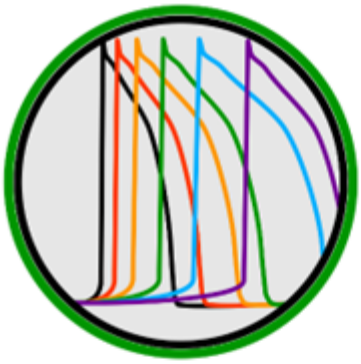


Interface

Once the app has launched, you will have access to a simplified interface with three main choices:

1. Select Spontaneously Beating or Paced
2. Select the APDs for which you want quantitative data. APD90 is default and mandatory.
3. Enter the number of APs that you want to be averaged at the steady state. The default is 5.
4. Enter the number of APs that you want to be used for SD1 and SD2 calculations. The default is 30, if available.
5. Spontaneous Beating only: Enter the minimum voltage threshold for automatic peak detection. -10 mV is the default value.
6. Spontaneous Beating only: Select either you want to save results of analysis for the entire file or just at steady state.

After entering all the parameters, click **RUN** to start the analyses.



Batch Action PoTential Analyser

Were the cells paced or spontaneously beating?

- (•) Spontaneously Beating
- ( ) Paced

Which Action Potential Durations you want? (APD90 is mandatory)

- [ ] 5
- [ ] 10
- [ ] 20
- [ ] 30
- [ ] 40
- [ ] 50
- [ ] 60
- [ ] 70
- [ ] 75
- [ ] 80
- [ ] 85
- [x] 90
- [ ] 95

How many sweeps you want to average for steady state? (Default = 5)

5

How many sweeps you want to average for SD1 and SD2 calculations? (Default = 30)

30

(Only for Spontaneously Beating) What is the minimum voltage threshold for automatic peak detection? (Default = -10 mV)

-10

(Only for Spontaneously Beating) Do you want to save/average all data or only the APs at the steady state?

- [x] SS
- [ ] All

Run! Stop

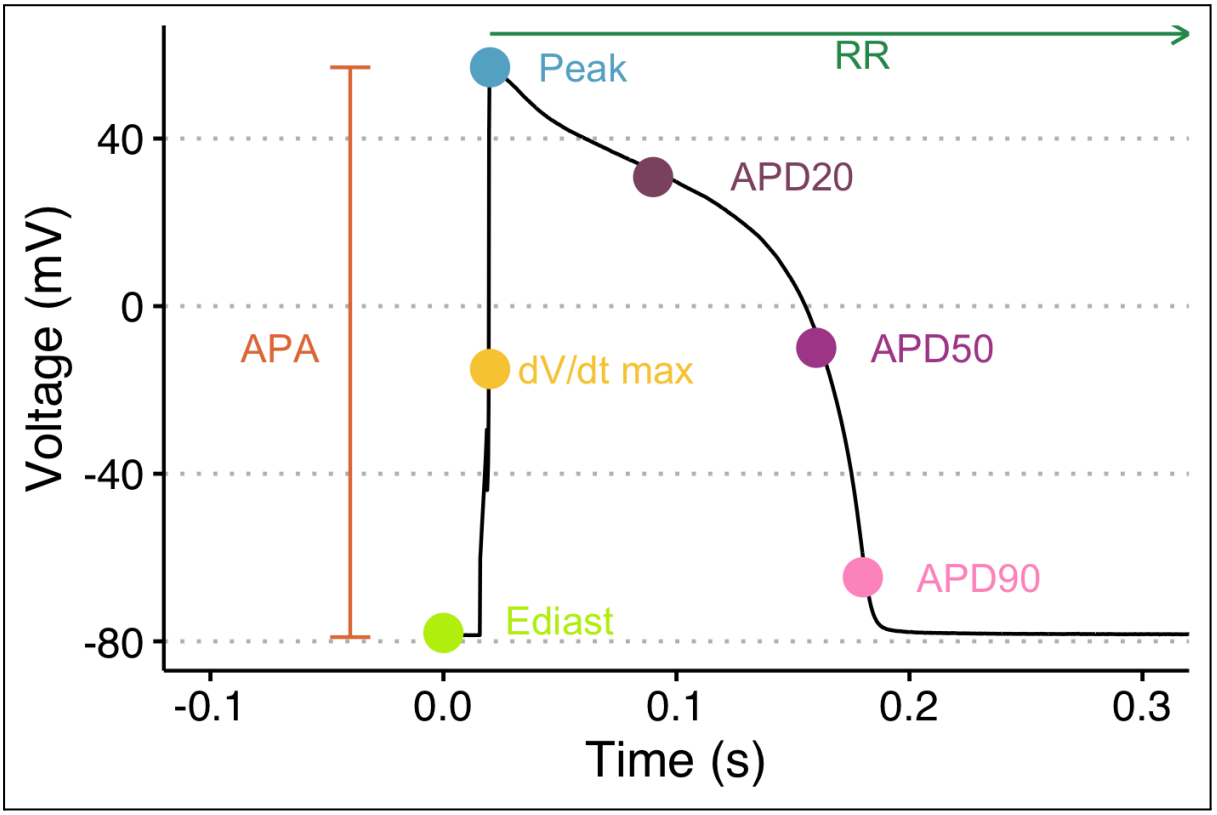
IMPORTANT: First attempt to run BAPTA will ask you permission to install required packages. You should give consent for installation.

Analyses

The tool will automatically create the analysis folders based on input folder names.

Parameters in output

Parameter	Abbreviation	Measure Unit	Description
AP Durations	APD	ms	AP durations (APD) at 10% to 90% of the repolarisation phase, from APD_{10} to APD_{90} in steps of 5% or 10%.
Resting Membrane Potential	E_{diast}	mV	Resting membrane potential.
AP Amplitude	APA	mV	The voltage span of the AP from the baseline to the peak.
AP Peak	Peak	mV	The maximum upstroke peak voltage value reached by the AP.
Maximum Upstroke Velocity	dV/dt_max	V/s	The maximum upstroke velocity of the depolarisation phase of the AP.
Maximum Decay Velocity	-dV/dt_max	V/s	The maximum velocity reached in the repolarisation phase of the AP.
Short Term Variability of the repolarization duration	SD1	-	The Short Term Variability of the APD90 as defined in [13,15].
Long Term Variability of the repolarization duration	SD2	-	The Long Term Variability of the APD90 as defined in [13,15].
RR Interval	RR	ms	The time distance between two consecutive AP peaks.



Analysis Folder Structure

```
.
└── output
    └── analyses
        └── `foldername`
            ├── APA
            │   ├── `filename` APA.csv
            │   └── ...
            ├── APD
            │   ├── `filename`.csv
            │   └── ...
            ├── dVdt_max
            │   ├── `filename` dVdt_max.csv
            │   └── ...
            ├── Ediast
            │   ├── `filename` Ediast.csv
            │   └── ...
            ├── Negative_dVdt_max
            │   ├── `filename` Negative dVdt_max.csv
            │   └── ...
            ├── Peak
            │   ├── `filename` Peak.csv
            │   └── ...
            └── `foldername` Mean Values.csv
```

Analysis Files

The tool will generate the following analysis files:

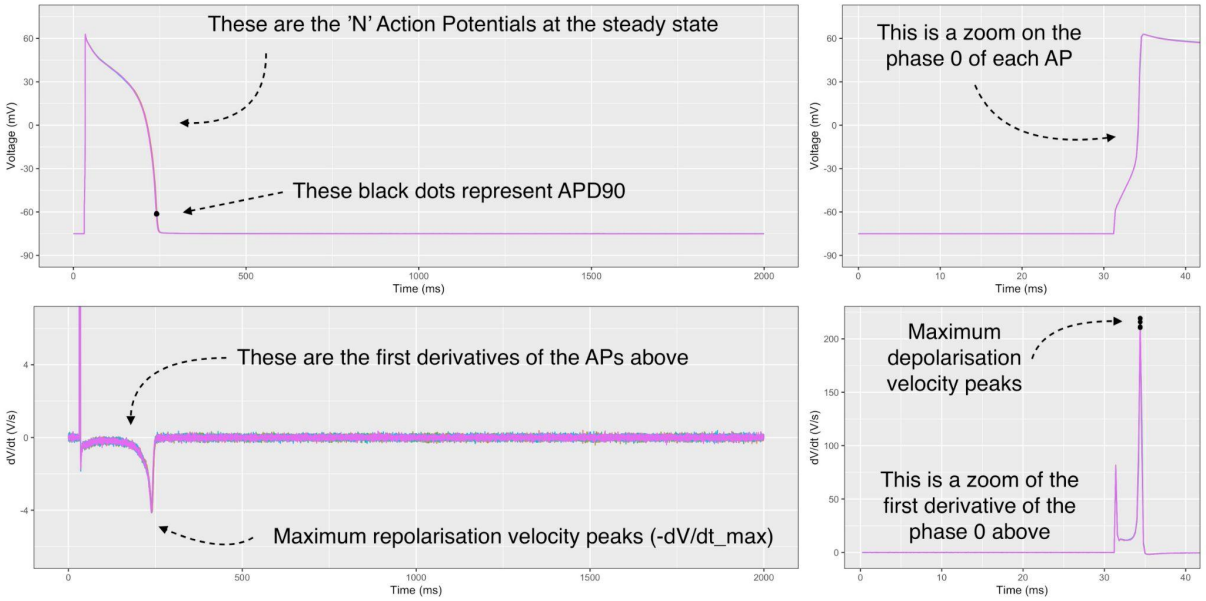
Experiment folder name: Mean Values.csv
(e.g. in folder “GP_CTR_PacingFrequency1” Mean Values.csv file)

This file contains the mean values for all the parameters, indicated above, for each of the analysed files. Specifically for APDs: according to the number of APD values chosen at the beginning of the analysis, more columns will be added to this table. If APD_10, APD_50, APD_90 are selected at the beginning, the table will add 3 columns.

Plots

The tool will generate the following plots:

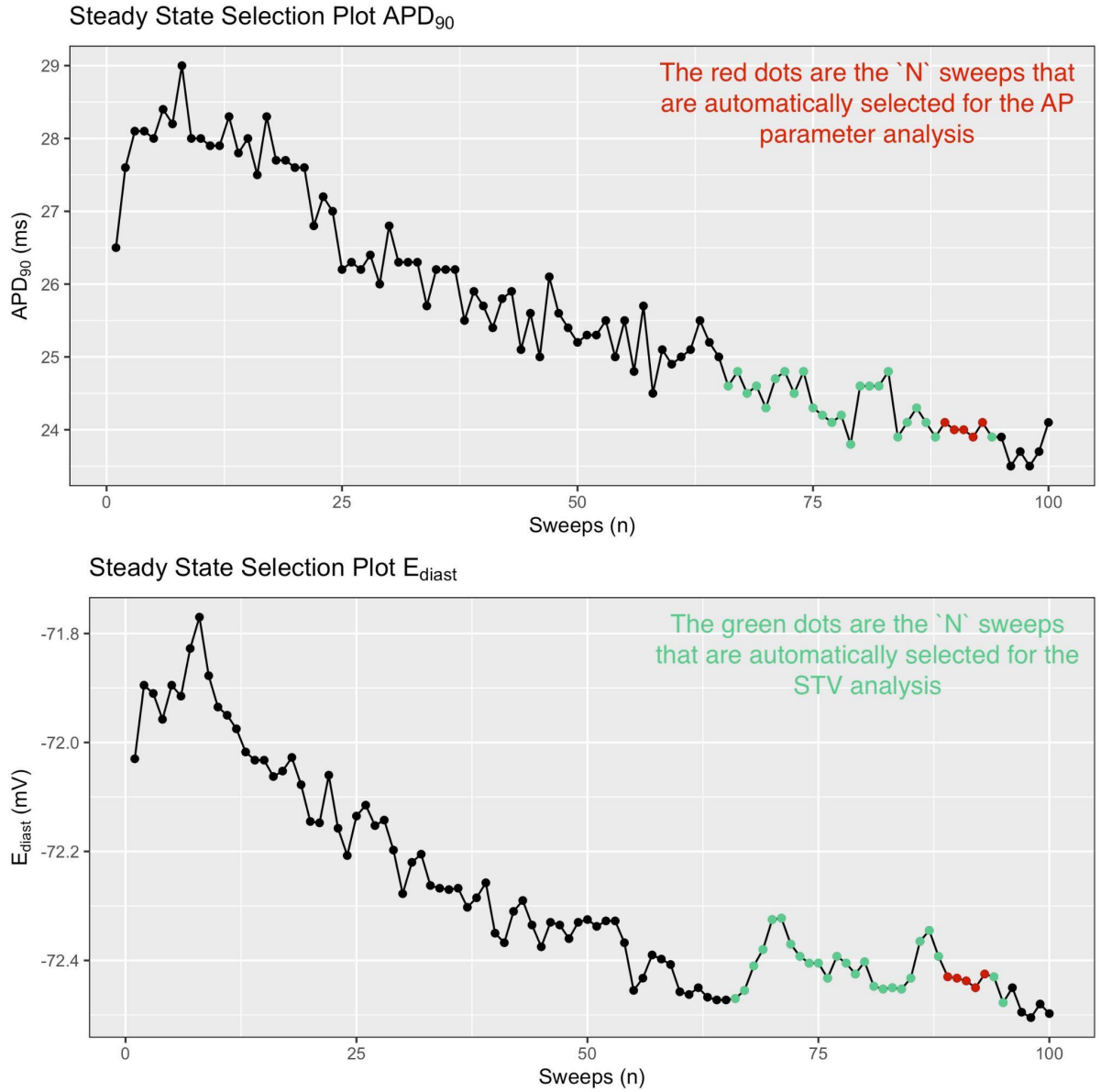
```
.
└── output
    └── img
        └── `foldername`
            ├── `filename` AP Parameters.jpeg
            ├── `filename` APD Values.jpeg
            ├── `filename` BVR_APD90.jpeg
            └── `filename` SS Selection.jpeg
```



This plot is made by 4 panels:

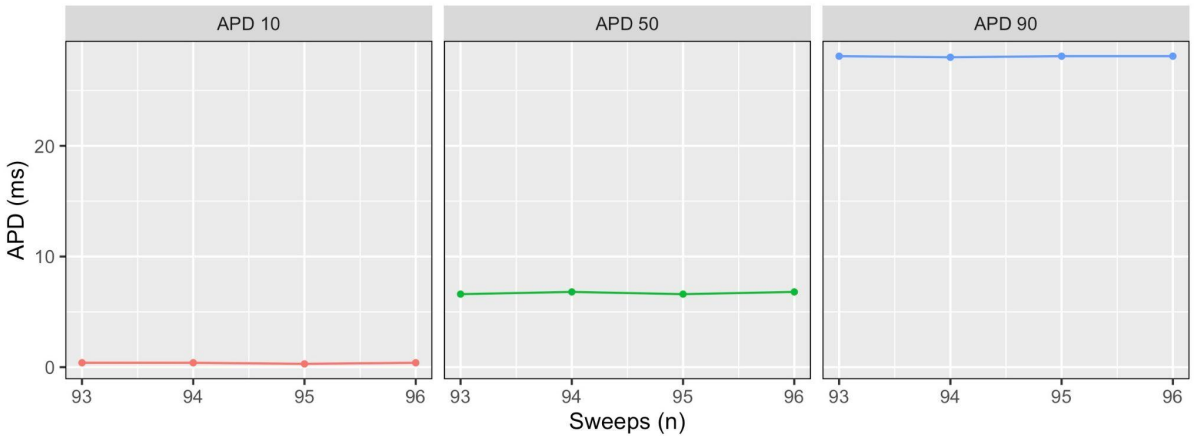
1. Top left: the representative APs automatically selected by the tool. The black dots represent the APD90 of each AP.
2. Bottom left: the first derivative of the aforementioned APs, which clearly shows the max. repolarisation velocity (-dV/dt_max).
3. Top right: this panel helps the user to see whether the upstroke phase is clear or whether it has an artifact due to the stimulus pulse.

4. Bottom right: the first derivative of the zoomed-in APs. This helps the user to understand whether the upstroke velocity (dV/dt_max) is correctly measured. Black dots indicate the dV/dt_Max points that have been used for the analyses.

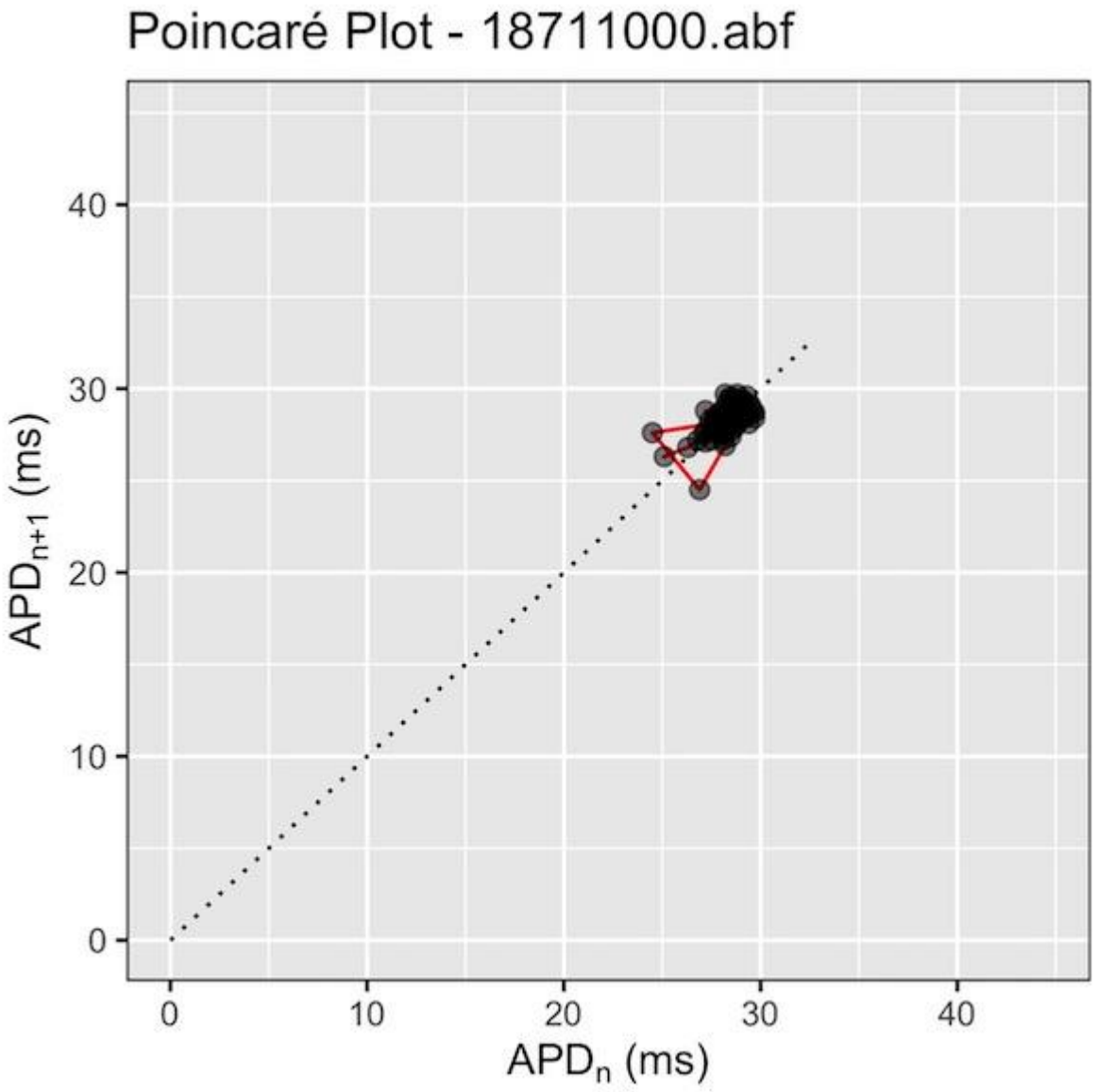


This plot is very important: it shows the time course of APD90 (top) and Ediast (bottom); coloured in red are the points automatically selected for the subsequent AP analyses, while in green are indicated those selected for the STV analysis. The automatic selection is based on two parameters:

The polarisation of E_diast: the tool will select the APs in the most negative quantile (default 80%) which should be as much negative as possible. This filter may not be very useful for adult CMs, but for stem cell-derived CMs it seems that more reliable APD values are obtained when negative Ediasts are used.
The variability of the APD90, which should be as minimum as possible for a proper steady state.



This plot may be useful to check whether all the selected AP values are in a steady state. It may also be useful to get APD values in a glance.



This is the Poincaré plot used to calculate and visualize APD90 dispersion (SD1 and SD2) as previously done in Altomare et al., Circulation: Arrhythmia & Electrophysiology, 2015.

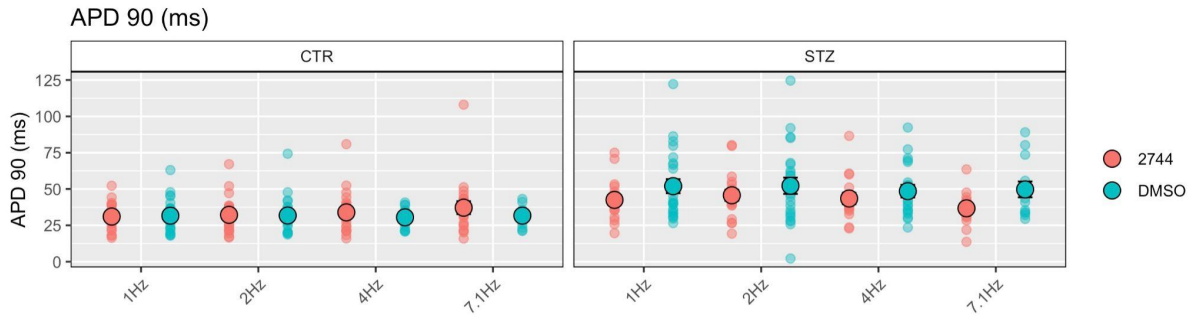
Summary plots

Once the script has finished, you may want to run the *“AP_Output_Analysis.R”* file in the *“scripts”* folder, which analyses all the average traces and plots them in bar graphs. This script will work only when the data have the same number of fields (check the Folder Structure section above) otherwise no comparison would be possible.

Each parameter is individually outputted and the following plots are generated:

```
.
└── output
    └── img
        ├── `APA_Averages.jpeg`
        ├── `APD_N*_Averages.jpeg`
        ├── `dVdt_Averages.jpeg`
        ├── `Ediast_Averages.jpeg`
        ├── `Negative_dVdt_Averages.jpeg`
        ├── `Peak_Averages.jpeg`
        └── `STV_Averages.jpeg`
```

For a more efficient group comparison, when more than one experimental group is analysed, the images will additionally include the "Combined" version, with groups and conditions compared on the same plot.



The automated output analysis provides a complete table with all the mean data and some basic graphical comparisons between groups.

Limitations

BAPTA cannot discriminate between normal or diseased action potentials, thus arrhythmic events occurring during phases 1-3 of the cardiac AP (e.g. EADs) are not currently recognised.
BAPTA can currently be used downstream to patch clamp recordings obtained from Molecular Devices hardware and the respective *.abf* files.