

# CorrectCoxVramp – a python function to correct for space-clamp errors in voltage-ramp experiments

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## Introduction

The voltage-ramp technique is used to estimate the dependence of a stationary membrane current ( $I$ ) on an applied voltage ( $V$ ). As the name implies,  $V$  is linearly ramped while  $I$  is recorded. It is assumed that the ramp is sufficiently slow so that at each point  $I$  is effectively stationary, i.e. if the ramp were stopped at some point  $I$  would not change (an assumption that can, of course, be tested experimentally). Hence the  $V$ -ramp provides no information about kinetics. Nevertheless, it is still useful for estimating the  $V$ -dependence of a pharmacologically isolated specific conductance  $g(V) = i(V)/(V-E)$ , where  $i$  is the current density (e.g.  $\text{mA cm}^{-2}$ ) and  $E$  is the reversal potential. However, a serious problem when applying the  $V$ -ramp technique to neurons is that  $V$  is clamped only at the recording site. This will often be the soma, or perhaps an axonal bleb or varicosity, although proximal dendrites can also be patched. Away from the recording site  $V$  will not be clamped at the desired values (“space-clamp error”), but the membrane current at those points will contribute to the current measured at the recording site. Hence the interpretation of the recorded values of  $I$  and  $V$  is not straightforward; rather one must in some way correct for this space-clamp error. Various approaches have been tried (reviewed briefly by Murphy *et al.*, 2023). Here we take as our starting point the method described by Cox (2008), which provides an initial estimate of  $i(V)$ . This is then improved upon iteratively. Full details of the method are given in Murphy *et al.* (2023). Here I describe the use of a Python function `CorrectCoxVramp`, which operates in conjunction with the simulator NEURON (Carnevale and Hines, 2009) to correct for space-clamp errors in  $V$ -ramp data and so return an estimate of  $i(V)$ . Only depolarising ramps (positive slopes) are supported at present.

## Using the function `CorrectCoxVramp`

Create a folder (which we will call the “input folder”) and put your NEURON files into it, together with a text file containing the  $V$ -ramp data. An example input folder (`CorrectCoxVrampExample`) is provided. The example ramp-data file is `NaP_Mean_IV.txt`; your data must conform to the data in that file (although the unit of current can be nA rather than pA; see below). Copy the file `CorrectCoxVramp.py` to your input folder. Next create a parameter file and save it to the input folder; you can use the provided `parameters.txt` as a template. The meaning of the parameters is given in the

Table 1. Put your own Python script(s) into the input folder and import `CorrectCoxVramp` by putting a line like the following near the start of your script:

```
import CorrectCoxVramp as cox
```

Calling the function from your script is simple; `result` is a dictionary:

```
result = cox.CorrectCoxVramp('parameters.txt')
```

An example script is provided (`CorrectCoxVrampExample.py`), together with an example NEURON model (the `.hoc` and `.mod` files in `CorrectCoxVrampExample`; remember to compile the latter). You can use any parameter file name you like, but parameter names and the colons in the parameter file must not be changed. As some parameters are needed by the NEURON model, these can be loaded from the parameter file by your hoc script (see `mossy_fiber_run.hoc`). You can also add your own text lines, but these must conform to the format in `parameters.txt` (e.g. “`neurite_id: 3`”). The name of the parameter file can be loaded by your NEURON model from `parameter_file.txt`. You may never need to use `result`, but in case you do its entries are given in Table 2.

**Table 1. Definition of parameters.**

| Parameter                        | Example                 | Description   |
|----------------------------------|-------------------------|---|
| <code>neuron_path</code>         | <code>C:/nrn/bin</code> | Omit this line if you want your system to find <code>neuron.exe</code> .                        |
| <code>neuron_file</code>         | <code>model.hoc</code>  | NEURON model file.  |
| <code>maxit</code>               | 9                       | Maximum number of iterations.   |
| <code>DV (mV)</code>             | 0.1                     | See $\Delta V$ and Eq. (17) in Murphy <i>et al.</i> (2023).                                     |
| <code>Iunit</code>               | nA                      | Unit of current in <code>tVIfile</code> (pA or nA; the latter will be converted to the former). |
| <code>Optimise_Cm</code>         | yes                     | yes = optimise $C_m$ and $i(V)$ ; otherwise $C_m$ is fixed.                                     |
| <code>Interpolation</code>       | modified                | Type of interpolation (see Technical Notes).  |
| <code>maxIerror_tol (pA)</code>  | 0.1                     | Maximum allowed error on $I$ .  |
| <code>Icap_error_tol (pA)</code> | 0.1                     | Maximum allowed error on the capacitive current ( $I_C$ ) if <code>Optimise_Cm</code> = yes.    |

|                          |           |  |
|--------------------------|-----------|--|
| V_LJP_corrected          | no        | Is $V$ corrected for the liquid junction potential?  |
| tVifile                  | Vramp.txt | V-ramp data ( $t$ , $V$ , $I$ ), e.g. NaP_Mean_IV.txt.   |
| coxdir                   | tempCox   | Temporary folder for intermediate data.  |
| outputdir                | output    | Output folder (absolute or relative); <b>will be deleted if it already exists.</b>                                     |
| Area (um <sup>2</sup> )  | 100       | Membrane area of isopotential compartment (e.g. soma, bleb, dendritic section); nseg = 1.                              |
| diam (um)                | 0.4 0.4   | List of neurite diameters (minimum 2).   |
| Ra (Ohm.cm)              | 100       | Axial resistivity.   |
| Raccess (MOhm)           | 10        | Patch access resistance.   |
| Rseal (GOhm)             | Inf       | Patch seal resistance.   |
| Cm (uF/cm <sup>2</sup> ) | 1.0       | Membrane capacitance (initial or fixed value).   |
| LJP (mV)                 | 2.0       | Liquid junction potential <sup>1</sup> .   |
| gL_fraction              | 0.01      | Use this fraction of $i(V)$ to estimate the leak conductance ( $g_L$ ) and reversal potential ( $E_L$ ) <sup>2</sup> . |
| VRm (mV)                 | -85 -65   | Estimate the specific membrane resistance ( $R_m$ ) over this $V$ range <sup>2</sup> .                                 |
| thold (ms)               | 200       | Pre-ramp holding interval.   |
| tstart (ms)              | 400       | V-ramp start time.   |
| poly                     | 2 3 -80   | Specifies the polynomial(s) used to smooth $I^3$ .   |
| do_plots                 | yes       | yes = do plots for each iteration.   |

<sup>1</sup>Used to calculate the leak current through the seal resistance,  $(V + \text{LJP})/R_{\text{seal}}$ .

<sup>2</sup>Not necessary but must be present; just ignore if don't want these results.

<sup>3</sup>Smoothing polynomial(s); `cox.test_smooth('parameters.txt')` plots the fit. See poly table on next page.

poly:

|         |   |
|---------|---|
| 3       | Fit a cubic polynomial to $I(V)$ and $I^*(V)$ (see Murphy <i>et al.</i> (2023) for the meaning of the latter).                                      |
| 4 4.0   | Find the ‘best-fit’ polynomial of degree $\leq 4$ using forward selection with an $F$ -to-enter value (float) of 4.0 (Kutner <i>et al.</i> , 2004). |
| 2 3 -80 | Splice together polynomials of degrees 2 and 3 at $V \approx -80$ mV (initial guess).   |
|         | Don’t fit a polynomial; use linear interpolation (not recommended).   |

**Table 2. Definition of entries in the `result` dictionary.**

| Key                          | Description  |
|------------------------------|--|
| <code>t (ms)</code>          | Sampling times for the $V$ -ramp data.   |
| <code>V (mV)</code>          | Observed $V$ values after correcting for $R_{\text{access}}$ (and LJP if necessary).     |
| <code>I (pA)</code>          | Observed $I$ values after correcting for $R_{\text{seal}}$ and capacitive current.       |
| <code>predI (pA)</code>      | Predicted $I$ values after correcting for $R_{\text{seal}}$ and $I_C$ . Pairs with $V$ . |
| <code>cd (mA/cm^2)</code>    | Estimated current density. Pairs with $V$ .  |
| <code>Cm (uF/cm^2)</code>    | Estimated membrane specific capacitance.   |
| <code>Icap_error (pA)</code> | Error on the predicted capacitive current ( $I_C$ ).                                     |
| <code>maxIerror (pA)</code>  | Maximum error on the predicted injected current.   |
| <code>done</code>            | 1 = done, 0 = not done.  |
| <code>Run time (s)</code>    | What it says.  |
| <code>Best iteration</code>  | Ditto.   |
| <code>output_folder</code>   | Ditto.   |

Results are also saved to the output folder; file names are given in Table 3. The parameter and V-ramp data files are also copied to the output folder. If you wish the temporary folder `coxdir` to be copied to the output folder, add an argument `saveCox = 'yes'` when calling `CorrectCoxVramp`. During the optimisation process, a number of temporary files are also created in the input folder. These files, which are described in Table 4, allow your NEURON program to interact with `CorrectCoxVramp`.

To run a series of optimisations using different parameter values (e.g. different  $R_a$  values or different fixed  $C_m$  values) put the call to `CorrectCoxVramp` in a loop, creating a new parameter file (containing a different output folder name) for each turn of the loop. E.g.

```
Ra = [110, 120, 130]; j = 0
file = open('parameters.txt','r')
paras = file.readlines(); file.close()
while j < len(paras):
    if 'Ra(Ohm.cm)' in paras[j]: jRa = j
    if 'outputdir' in paras[j]: jout = j
    = j + 1
i = 0
while i < len(Ra):
    print('')
    print('Ra(Ohm.cm): '+str(Ra[i]))
    outputdir = 'output_Ra='+str(Ra[i])
    paras[jRa] = 'Ra(Ohm.cm): '+str(Ra[i])+'\n'
    paras[jout] = 'outputdir: '+outputdir+'\n'
    parafile = 'parameters_Ra='+str(Ra[i])+'.txt'
    file = open(parafile,'w')
    file.writelines(paras); file.close()
    result = cox.CorrectCoxVramp(parafile,saveCox = 'yes')
    i = i + 1
```

In principle you could also use an optimiser to find the best value of  $R_a$  etc., but in practice I haven't been able to get this to work.

## Technical notes

As discussed in Murphy *et al.* (2023), the method involves the solution of a nonlinear ODE in the current density,  $i$  (their Eq. 13). Specification of the ODE requires estimation of a fictitious current ( $I^*$ ), which is determined by linear interpolation (or extrapolation if this is not possible). Possible choices for the Interpolation method in the parameter file are simple, standard and modified, where the latter two are taken from Gerald and Wheatly (1984). In principle these should be more robust than simple. Also modified is faster than standard and so is the recommended method. The initial value  $i = 0$  is taken at  $V = V_{\text{rest}}$ . In principle there can be multiple  $V_{\text{rest}}$  values but they must be stable equilibrium points (i.e.  $dI/dV > 0$  for  $I = 0$ ). If necessary such points are manufactured by linear extrapolation from the beginning and/or end of the  $I(V)$  data. If an unstable equilibrium point is present ( $dI/dV < 0$  for  $I = 0$ ) it is excluded from the analysis. Eq. (13) is then solved for points to the left and right of the unstable point, followed by concatenation of the two solutions to obtain the final result for  $i(V)$ . Eq. (13) is solved for  $i(V)$  using function `scipy.integrate.solve_ivp` from the *SciPy* Python package. Depending on the setting of `poly`,  $I(V)$  and  $I^*(V)$  are smoothed using the *SciPy* function `optimize.least_squares`, or the *NUMPY* function `polyfit`, or linearly interpolated using the *SciPy* function `interpolate.interpld`. Values of  $V_{\text{rest}}$  are estimated as the roots of the fitted functions, obtained with the function `numpy.roots` for the polynomials or by linear interpolation. All analyses are performed on the smoothed or interpolated data. To check the fit of the polynomial(s) to the raw ramp current, call `cox.test_smooth('parameters.txt')` and if necessary adjust `poly` in the parameters file before calling `CorrectCoxVramp`.

**Table 3. Output files names. # is the best iteration number.**

| File name                     | Description  |
|-------------------------------|--|
| cd_#.txt                      | Estimated $i(V)$ in two columns ( $V$ , $i$ ).   |
| CmRmL_#.txt                   | Estimates of $C_m$ , $R_m$ , $g_L$ and $E_L$ .   |
| IterationTimeErrors_#.txt     | Run time and errors ( $I$ and $I_C$ ).   |
| printout.txt                  | Run time, errors and $C_m$ for each iteration.   |
| Ierror_#.txt                  | Prediction errors for $I$ at each $V$ .  |
| IcapIholdVholdVrest.txt       | Estimate of $I_C$ , $I_{hold}$ , $V_{hold}$ and $V_{rest}$ .   |
| PredIcapIholdVholdVrest_#.txt | Predicted values of $I_C$ , $I_{hold}$ , $V_{hold}$ and $V_{rest}$ .   |
| Raw_tVI.txt                   | Raw observed $t$ , $V$ , $I$ (excluding pre-ramp data).  |
| tVI.txt                       | Corrected observed $t$ , $V$ , $I$ (excluding pre-ramp).   |
| Raw_tVpredI_#.txt             | Raw predicted $t$ , $V$ , $I$ (excluding pre-ramp).  |
| tVpredI_#.txt                 | Corrected predicted $t$ , $V$ , $I$ (excluding pre-ramp).  |
| Pred_fn_#.txt                 | Raw predicted $t$ , $V$ , $I$ (including pre-ramp), where $fn$ is the name of the $V$ -ramp data file ( <code>tVIfile</code> in the parameter file, excluding the “.txt”). |

The image files `Raw_I_and_I_corrected_for_Rseal_and_Cm.png`, `Current_density.png` and `Effect_of_an_access_resistance_on_V.png` contain what their names suggest.

**Table 4. Temporary files in the input folder.**

| File name   | Description  |
|-------------|--|
| tVpredI.txt | Input for CorrectCoxVramp. Save your NEURON-model predictions ( $t$ , $V$ , $I$ ), including pre-clamp holding data, to this file. The format must conform to that of tVpredI.txt in CorrectCoxVrampExample.   |
| flag.txt    | <p>Input for CorrectCoxVramp. Create this file with the following hoc code (or equivalent) when your NEURON program has finished running.</p> <pre>objref file proc finish() {     file = new File()     file.wopen("flag.txt")     file.printf("Done")     file.close()     quit() }</pre> <p>Anything other than "Done" will cause CorrectCoxVramp to terminate.</p>   |
| cd.txt      | Input for your NEURON model. The current best estimate of the current density as a function of $V$ , $i(V)$ , in two columns ( $V$ , $i$ ). Pass these data as a FUNCTION_TABLE to your .mod file (see cd.txt and the .hoc and .mod files in folder CorrectCoxVrampExample). Note that $i(V)$ includes the leak current density, $g_L(V - E_L)$ .  |
| CmRmL.txt   | <p>Input for your NEURON model. The current best estimate of <math>C_m</math> (<math>\mu\text{F cm}^{-2}</math>). This will not change from the value set in the parameter file if Optimise_Cm = no. Import it with something like:</p> <pre>objref file strdef s { file = new File() file.ropen("CmRmL.txt") } { file.scanstr(s) m = file.scanvar() } file.close()</pre> <p>Additional lines in CmRmL.txt contain estimates of <math>R_m</math>, <math>g_L</math> and <math>E_L</math>.</p> |



## Acknowledgements

The morphology of the neuron model in mossy\_fiber\_morphology.hoc was based on *hoc* code written by Konstantin Ostroumov (<https://prabook.com/web/konstantin.ostroumov/476287>).

This software was developed in the laboratory of Prof. Johan F. Storm at the University of Oslo with financial support from the European Union's Horizon 2020 research and innovation programme under grant agreement 7202070 (Human Brain Project; HBP, SGA3) and the Norwegian Research Council (NFR/SFF).

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