ReScience

[Re] Chemical Signalling in the Neurovascular Unit

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Article repository

Code repository

Data repository

A reference implementation of

→ Witthoft A, Karniadakis GE (2012) A bidirectional model for communication in the neurovascular unit. *Journal of Theoretical Biology* 311: 80-93.

Introduction

Witthoft and Karniadakis [1] introduced a model for communication via chemical signalling within the neurovascular unit (NVU). In the NVU neurons communicate their nutrient requirements to the cerebral arteries via astrocytes [3]: the activity of neurons releases glutamate and potassium into the synaptic space, causing astrocytes to release potassium into the periarterial space via their end-feet, leading to the activation of smooth muscle cells in the artery wall and thus a dilation of the artery to increase blood flow.

Functional hyperemia is an important mechanism in the brain, by which an increased neuronal metabolism leads to an increase of blood flow in surrounding arteries in order to maintain adequate oxygen and nutrient supply to the active neurons. This process works via a cascade of chemical signalling processes from neurons to astrocytes to arteries. The unidirectional vascular response to increased neuronal demands has previously been modelled [7][5], however, these models did not include the activity of the transient receptor potential vanilloid-related channel 4 (TRPV4). The TRPV4 channel is a mechanosensitive calcium channel and leads to a depolarisation of the astrocyte in response to the dilation of the artery wall.

The paper contains the differential equations that are being modelled, however no code repository is provided. The model is useful for the investigation of the effect of cerebrovascular diseases on the brain and thus an openly available code repository reproducing the results of this paper will be helpful to other researchers.

Methods

The paper indicates that the original code was written in Matlab, while the reproduction presented here is written in Python 3.5. The model is given as a series of ordinary differential equations (ODE), which are implemented as a function nvu(). This function is solved using the scipy.integrate library. Initial attempts to solve the system of ODE using the standard function odeint() failed. Instead, the equations can be solved using the function ode() with the integration method set to "lsoda", which automatically selects between the Adams method for non-stiff and BDF for stiff problems. The ODE system described in [1] is a stiff system. Values for absolute and relative tolerance have to be set in order for the simulation to finish successfully.

These are given in the results section as they differ for different simulations. Future users of this code should note that the simulation of a longer time span may require the use of different values for absolute and relative tolerance, which have to be found via trial and error, but from experience, they should not be larger than 1e-4 or smaller than 1e-11.

The code was implemented following the descriptions of the equations in [1], but upon cross-checking equations against their equivalents in [7][5][6] it became evident that some of the equations contain typographical errors. This was confirmed after contacting the authors of [1]. Changes to the equations are as follows:

- $I_{\Sigma K}$ in (20) should be defined as $I_{\Sigma K} = -J_{\Sigma K} C_{astr} \gamma$ (changed sign).
- (23) J_{Ca} is not defined in the manuscript, but correspondence with the original authors revealed that it should be defined as $J_{Ca} = -I_{Ca}/(C_{SMC}\gamma)$.
- (23) Both J_{TRPV} and J_{Ca} should be divided by the volume ratios of perivascular to astrocyte and SMC space (analogous to equation (22)), such that it reads $\frac{d\left[Ca^{2+}\right]}{dt} = -\frac{J_{TRPV}}{VR_{pa}} - \frac{J_{Ca}}{VR_{ps}} - Ca_{decay}\left(\left[Ca^{2+}\right]_p - \left[Ca^{2+}\right]_{p,0}\right)$.
- (31) $I_K = g_K n(V_m - v_K)$ [5].
- (A.1) Term $\frac{1}{2\alpha} I_{Ca}$ should be αI_{Ca} (to comply with code for [2] by the original authors).
- (A.3) When implementing this equation it has to be multiplied by μm to get the correct units of force.
- (A.6) The brackets around the exponential function are set incorrectly, it should read $\exp\left(\frac{-(y'-y'_0)^2}{2\left[y'_1/(y'+y'_2)\right]^{2y'_4}}\right) - y'_3$ [6].
- Parameter C_{SMC} is given as C in Table B2 in [1]
- Decimal of parameter a in Table B2 in [1] is in the wrong place, it should read $a = 502.65\ \mu m^2$
- The value for $[\mathrm{Ca}^{2+}]_{\mathrm{ER}}$ is not given, but can be found in [7] as $[\mathrm{Ca}^{2+}]_{\mathrm{ER}} = 400 \mu m$
- Parameter $g_{TRPV} = 50$ pS is incorrectly cited from [4], correct value was found in the newer model [2]
- Parameter $k_{Ca} = 135.68$ 1/s is incorrectly cited from [6]

The input to the model is not explictly provided in the original publication, only the statement "We simulated neural-induced vasodilation by representing the total synaptic activity in the astrocyte domain as a uniform, continuous smooth pulse of glutamate (J_{K_s}) and of synaptic potassium ($[\mathrm{K}^+]_s$)." is provided [1]. Upon correspondence with the authors both glutamate and synaptic potassium were modelled as a linear increase over time up to a maximum value, which was sustained for a time period, followed by a linear decrease. The functions for the input are provided in the code of this implementation (Figure 1). Maximum values were found by trial and error such that potassium and IP_3 in Figure 2 here match Figure 5 in the origial publication.

Furthermore, upon correspondence with the original authors, an equilibration phase of 20 s before the simulation had to be implemented. During the equilibration phase no glutamate or potassium is released into the synaptic space and the solution values at the end of the equilibration phase is used as initial values for the main simulation. This step is not mentioned in the original publication, but is vital to obtain the correct results.

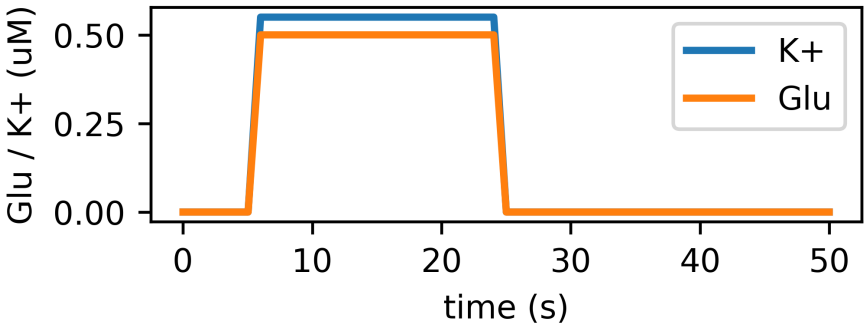

Figure 1: Input functions for the NVU model. K+ (blue) refers to potassium and Glu (orange) refers to glutamate released into the synaptic space as a consequence of synaptic activity by the neuron. Potassium levels are given in units of μM while the glutamate value actually refers to the ratio of bound and unbound glutamate receptors (equation (3) [1]) and is therefore unitless. Maximum values were found by trial and error such that potassium and IP_3 in Figure 2 here match Figure 5 in the original publication.

Results

Results from the code presented in this repository aim at reproducing Figure 5 in the original application, which shows ion and voltage dynamics over a simulation period of 50 s.

Figure 2 shows the results obtained from the code in this repository. Potassium (K^+) in both the synaptic and perivascular space match their corresponding plots in the original publication, as do calcium (Ca^{2+}) in the smooth muscle cell and the arterial radius. Differences can be seen for Ca^{2+} in the astrocyte, EET, the astrocyte membrane potential V_k and the perivascular K^+ concentration. In the case of astrocytic Ca^{2+} and EET the first peak overshoots compared to the results in the original publication, while the remainder of the plots match the original publication. Most interesting is the shape of V_k, which in the first half looks very different from the reproduced figure. The depolarisation as well as the relaxation after stimulus removal appear to occur much faster compared to the original publication. The depolarisation shows a small dip at around 7 s. Additionally, V_k overshoots into positive values, which is not given in the original publication. Perivascular K^+ does not reach 20 mM as in the original publication, which may be a result of the other differences.

The large difference in V_k between the two simulations is surprising, especially because this does not lead to incorrect results in the other plots, apart from an overshoot of the values for Ca^{2+} and EET in the astrocyte. The key results, which are the response of Ca^{2+} and subsequently the arterial radius match the original publication. A newer publication by the same authors exists [2], which does contain the same spike for Ca^{2+} and EET as the simulation results presented here. It is unclear, where this difference comes from as [6] does not indicate that any of the equations affecting Ca^{2+} or EET have been altered. It is also unclear why V_k shows such a large difference.

One possible explanation could be that some components of $\partial V_k/\partial t$ are wrongly implemented, but the results are mostly affected by the mere fact that V_k increases in response to the stimulus instead of the exact value of V_k. To test this, V_k was obtained from the original publication and hard-coded into the simulation instead of being calculated from its ODE. This is shown in Figure 3. Here, the results for K^+ in the perivascular space, Ca^{2+} in the smooth muscle cells and the arterial radius differ largely from the results in the original publication or indeed Figure 2. Because of this discrepancy between results the authors of the original publication were contacted to request the code for this publication. However, they could only provide the Matlab

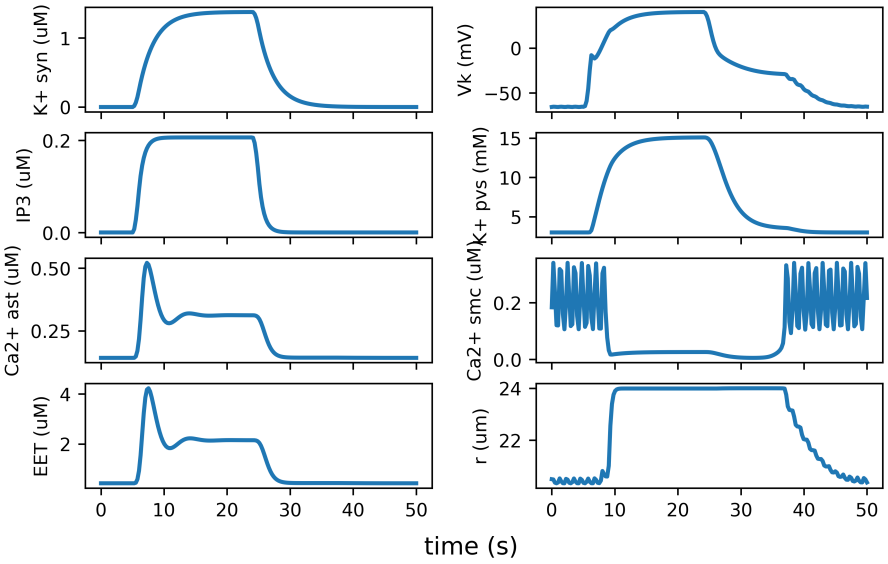

Figure 2: Astrocyte and arterial response during neuronal stimulation. This figure is a reproduction of Figure 5 in the original publication using the code in this repository. Potassium (K^+) in both the synaptic and perivascular space match their corresponding plots in the original publication, as do calcium (Ca^{2+}) in the smooth muscle cell and the arterial radius. Differences can be seen for Ca^{2+} in the astrocyte, EET and the astrocyte membrane potential V_k. In the case of astrocytic Ca^{2+} and EET the first peak overshoots compared to the results in the original publication, while the remainder of the plots match the original publication. Most interesting is the shape of V_k, which in the first half looks very different from the reproduced figure. Additionally, V_k overshoots into positive values, which is not given in the original publication. Simulation tolerance values atol=1e-7, rtol=1e-7.

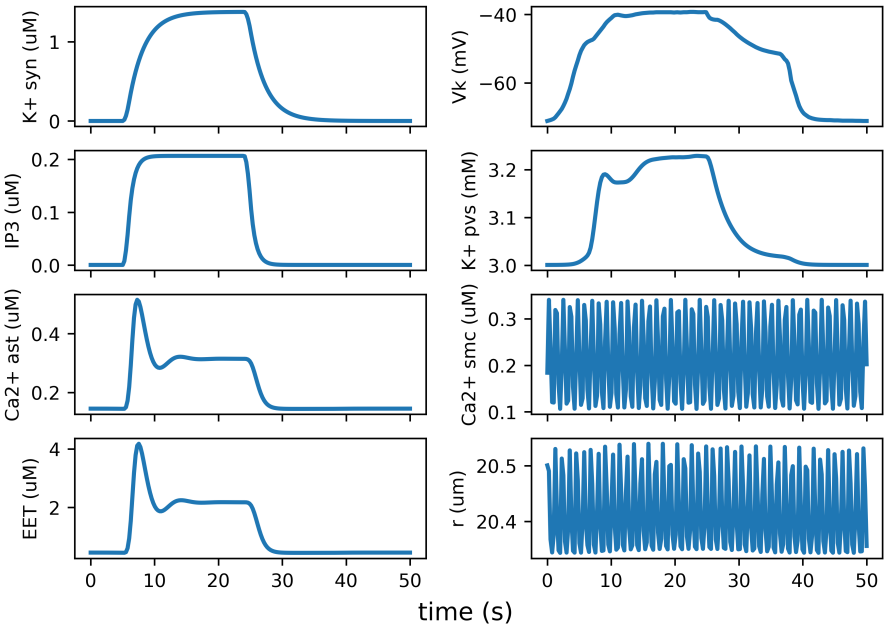

Figure 3: Astrocyte and arterial response during neuronal stimulation whilst V_k is fixed to match the original publication. The difference in results for K^+ in the perivascular space, Ca^{2+} in the smooth muscle cell and arterial radius suggest that possible further errors in the equations exist in the original publication. Simulation tolerance values atol=1e-7, rtol=1e-7.

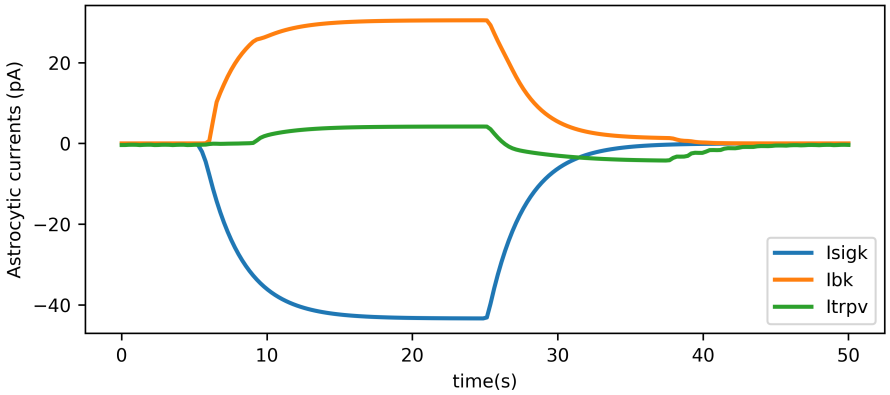

Figure 4: Astrocyte currents from equation (20). Comparing the astrocyte currents here to the ones presented in Figure S2 in [2] shows discrepancies. The shape of Isigk ($I_{\Sigma K}$, equation 20) matches (note that the widths of the figures differ), however its values differ by two orders of magnitude. Ibk (equation 15) appears to mostly match its equivalent in Figure S2. The shape of Itrpv shows similarities, but similar to Isigk the values are two orders of magnitude larger.

code for [2], from which it was not possible to revert back to the code to implement [1]. Attempts were made to remove the additional equations introduced in [2] from this code in order to revert it back to what it might have been at the time of publication of [1]. However, the results did not match. The original authors were asked for advice, but stated that it would likely be impossible to revert the code back to its original version due to the sensitivity of parameters, which would make this processes immensely error prone.

The supplementary information of the newer publication [2] contains plots of the astrocyte currents, which may help to find the differences between this implementation and [1] (Figure S2 [2], Figure 4). Comparing the astrocyte currents here to the ones presented in Figure S2 in [2] shows discrepancies. The shape of $I_{\Sigma K}$ (equation 20) matches (note that the widths of the figures differ), however its values differ by two orders of magnitude. I_{BK} (equation 15) appears to mostly match its equivalent in Figure S2. The shape of I_{TRPV} shows similarities, but similar to $I_{\Sigma K}$ the values are two orders of magnitude larger. Figure S2 in [2] contains additional KIR channel currents at the astrocyte, which are not included in the original paper. Vice versa, the leak channel equation has been removed in the newer model (most likely because this has been replaced by two explicit KIR channel equations). Thus, these are not plotted in Figure 4. $I_{\Sigma K}$ is mostly defined by the values for potassium in the synaptic space (equation 2). Because there is a discrepancy of the potassium values by three orders of magnitude between the two publications by Witthoft et al., this is the likely source for the discrepancy in values for $I_{\Sigma K}$. Only small differences exist in the parameter definitions for I_{BK}, but the size of the discrepancy between the two models also suggests that it may arise from the difference in potassium values at the input of the model. These differences make a direct comparisons between astrocyte currents difficult at best. Unfortunately, no results for the currents on the smooth muscle cell membrane (V_m, equation 30) are provided in either publication.

The original publication contains results to validate their TRPV model equations (Figure 3 [1]). They obtained data of the astrocyte membrane potential (Vk) of an artery being inflated by 20-40% of its original size in quick bursts [8]. The data of the stretching of the vessel was extracted from [1] and supplied as an input (variable x) to the NVU model. Figure 5 shows the equivalent of Figure 3 in the original publication. The figures compare well to some degree, however, the astrocyte membrane potential appears to decay too quickly in this simulation. Additionally, the value range does not match, which should be -72 mV to -40 mV in the original publication, while it is

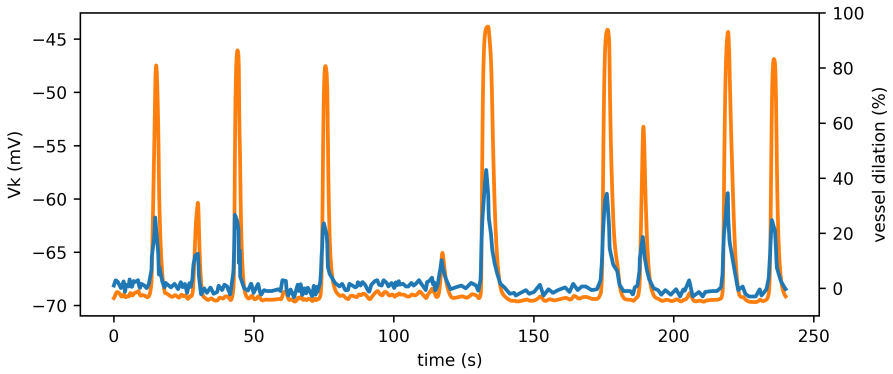

Figure 5: Astrocyte membrane potential due to quick manual inflation of the artery. The experimental data describing the artery stretch was obtained from [1] and originates from [8]. The astrocyte membrane potential was expected to range from -80 mV to -40 mV and its decay was expected to be slower.

-70 mV to approximately 25 mV here. Tests to vary the parameters of equation (23), which governs the change in perivascular calcium, did not yield further improvements.

Conclusion

To a large degree the results from [1] were replicated. However, distinct differences in the results remain, which could not be explained. It should be emphasised that the number of errors found in the original publication may indicate that more errors may remain to be found, however, because no version of the code to implement [1] appears to exist anymore, it will be very difficult at best to find these additional errors.

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