Evaluating Single and Multi-Neuronal Dynamics under Ischemic Conditions

Hristos Courellis
Department of Bioengineering
UC-San Diego
La Jolla, CA 92093

Daril Brown II
Department of Bioengineerng
UC-San Diego
La Jolla, CA 92093
Debrown@eng.ucsd.edu

Kaushik Sridhar
Department of Bioengineering
UC-San Diego
La Jolla, CA 92093

Abstract

It is necessary to examine the membrane dynamics of the neuron under ischemic conditions to understand the physiological changes that occur during metabolic perturbations. This would have far-reaching effects on exploring the brain metabolic activity following trauma. Any tissue in the body which has a metabolic demand requires the substrates for metabolism to be delivered, typically by the circulatory system. Of these substrates, molecular oxygen provides a means for cells to undergo aerobic respiration, which provides an abundance of ATP for further cellular activity. Our model is based on the single neuron approximation of the energy depleted state which exists under ischemic conditions. The original model was proposed by Zandt, first author of the reference paper Zandt et al. “Neural Dynamics during Anoxia and the ‘Wave of Death’”. This model features the dynamics of a single neuron operating under reduced depolarization conditions as a result of dynamic changes in the membrane potential and equilibrium concentrations of Na^+ and K^+ as a result of the reduced capacity of the ATP pump. The model we will be considering is strictly Hodgkin Huxley, since we need to consider the individual movements of sodium and potassium, and reduced models often eliminate the distinction between these variables to apply dimensionality reduction. For the small model simulations, we will evaluate dynamics of one and two neuron networks under ischemic conditions. In addition, we will investigate the effects of restoration of oxygen and glucose on our ischemic model to investigate the vitality of the neuron post-ischemia.

1 Introduction

Neurons are highly aerobic cells, which is why they are highly susceptible to irreparable damage during situations where their Oxygen supply is reduced or halted, called ischemia. If these conditions were to occur in the brain, such as in the case of a stroke, it is understandable that the result would be catastrophic. Currently the clinical treatment for acute & chronic ischemia is to return blood flow to the affected area as soon as possible. It is necessary to understand the effect of ischemia on Neurons and to learn at what point the damage done to the brain tissue is irreversible.

1.1 Aerobic Demand of Neurons

Neurons aerobic demand stems from their need to produce a usable form of energy, namely ATP, to perform maintenance and synaptic functions. This process depends on the circulatory system to provide molecular oxygen to the neurons so that they can undergo aerobic respiration, the process by which ATP is produced. A large volume of ATP is needed by neurons, because their NA^+/K^+ restoring pumps require ATP to restore membrane potential after every action potential conducted down the axon. Ischemia depletes the oxygenation of neurons which prevents large-scale generation of ATP. Without this restorative pump the neuron ceases to function properly.

1.2 Ischemic Conditions and the Wave Death

The negative effects of oxygen and glucose deprivation due to ischemia are apparent almost immediately after blood flow is cut off. This dysfunction was physiologically observed using electroencephalogram (EEG) as an increase in slow wave activity followed by complete cessation of activity. A slow wave lasting approximately 5–20 seconds appears after half a minute of electrocerebral silence. This wave was named the “Wave of Death” by Zandt, the first author of the reference paper Zandt et al. “Neural Dynamics during Anoxia and the ‘Wave of Death’”. It is thought to reflect the synchronous death of brain neurons.

2 Methods and Results

2.1 Single neuronal response to complete Oxygen-Glucose deprivation (OGD)

Python was used to model the action potential propagation and ion dynamics in a single Hodgkin-Huxley neuron under complete metabolic deprivation. We chose to start at the single neuronal level to try and replicate the “Wave of Death” phenomenon reported by van Rijn et al, PLoS One 6, e16514, 2011[1], wherein a slow depolarizing wave was observed in rats after euthanization. The authors hypothesized that this phenomenon could potentially serve as a biomarker for irreversible damage to the neuron. Using the Hodgkin-Huxley neuronal model, we modeled the underlying biophysical mechanism behind the slow depolarizing membrane potential. The Cressman model [2] was used to the estimate the ion dynamics of sodium, potassium and chloride ions under severe duress following oxygen-glucose deprivation.

$$C\frac{dV}{dt} = -I_{Na}(m_\infty(V), h, V - E_{Na}) - I_K(n, V - E_K) - I_{Cl}(V - E_{Cl})$$

$$where\ I_{Na}, I_K\ and\ I_{Cl}\ denote\ total\ sodium, potassium\ and\ chloride\ currents\ respectively.$$

The Cressman model used assumes dynamic intra-and extra-cellular concentrations for sodium, potassium and chloride ions.

$$\frac{d[Na]_i}{dt} = \frac{A}{VF}\left(-I_{Na} - 3I_p\right) \qquad \frac{d[K]_i}{dt} = \frac{A}{VF}\left(-I_K - 2I_p\right) \qquad \frac{d[Cl]_i}{dt} = 0$$

$$\frac{d[Na]_e}{dt} = \frac{\beta A}{VF}\left(-I_{Na} - 3I_p\right) \qquad \frac{d[K]_e}{dt} = \frac{-\beta A}{VF}\left(-I_K - 3I_p\right) - I_g - I_d \qquad \frac{d[Cl]_e}{dt} = 0$$

$$I_p = \left(\frac{\rho_p}{1 + e^{\frac{25 - [Na]_i}{3}}}\right) \times \left(\frac{1}{1 + e^{5.5 - \frac{[K]_e}{1}}}\right)$$

$$I_g = \left(\frac{G}{1 + e^{\frac{18 - [K]_e}{2.5}}}\right)$$

$$I_d = \in ([K]_e - k_\infty)$$

Apart from the ionic currents originating due to concentration gradients, we included 3 other sources of current namely Sodium-Potassium ATPase current (I_p), glial current (I_g) which serve as reservoir for extracellular potassium and diffusion current (I_d) of the glial potassium into the blood. We also included a factor (β) which includes the amount of volume occupied by a neuron in relation to the extracellular volume and a conversion factor to convert the current terms to concentration ($\frac{A}{VF}$). The rate of chloride ions were set at zero based on the average chloride migration in the cerebrospinal fluid of healthy human beings [3]. G signifies glial buffering rat and Є is diffusion rate.

Steady state value of variables is [4]:

Variable	**Steady state**	**units**
Vm	-68	mV
$[K]_i$	139	mmol
$[K]_e$	3.8	mmol
$[Na]_i$	20	mmol
$[Na]_e$	144	mmol
$[Cl]_i$	6	mmol
$[Cl]_e$	130	mmol

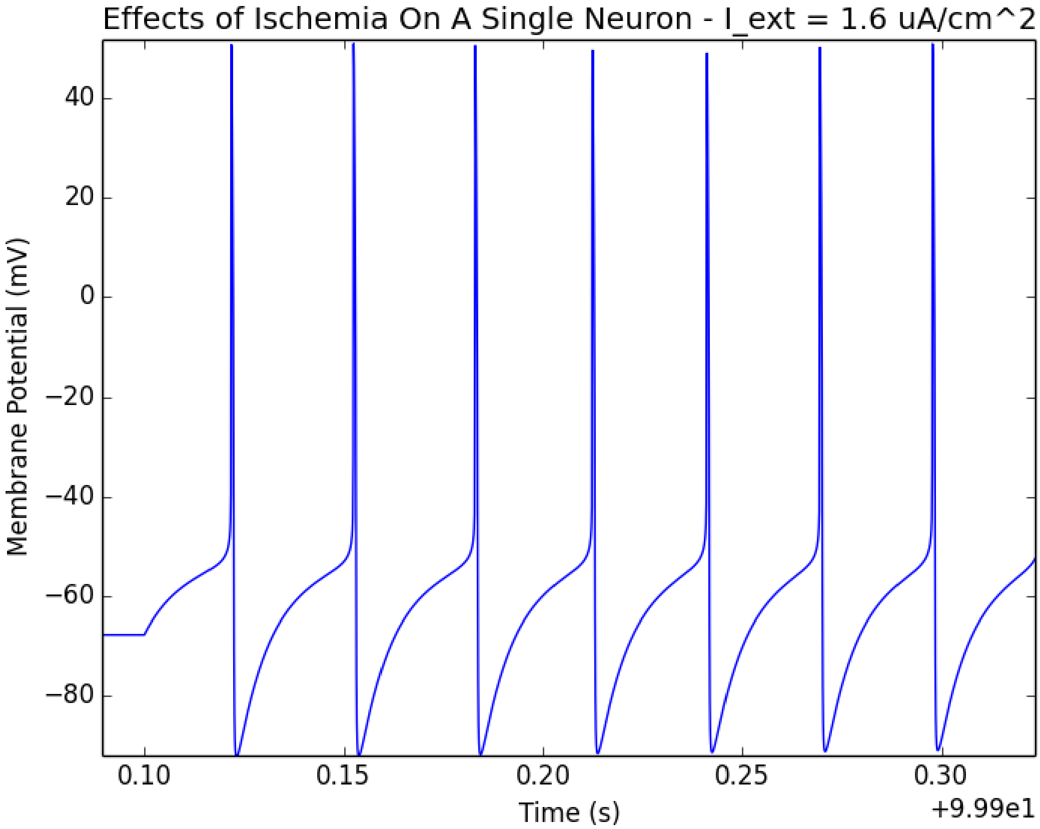


Figure : Membrane potential prior to complete anoxia shows a normal waveform exhibited by HH Neurons

Conditions for modeling complete OGD

Complete OGD is simulated by setting the pump current and the potassium uptake current by glial cells to zero. Due to this, the diffusion of potassium into the blood is also zero [4]

Due to this the ion dynamics vary as:

$$\frac{d[X]_e}{dt} = \frac{-\beta A I_x}{V Z_x F} \qquad \frac{d[X]_i}{dt} = \frac{-A I_x}{V Z_x F}$$

Where x is sodium, potassium or chloride ions.

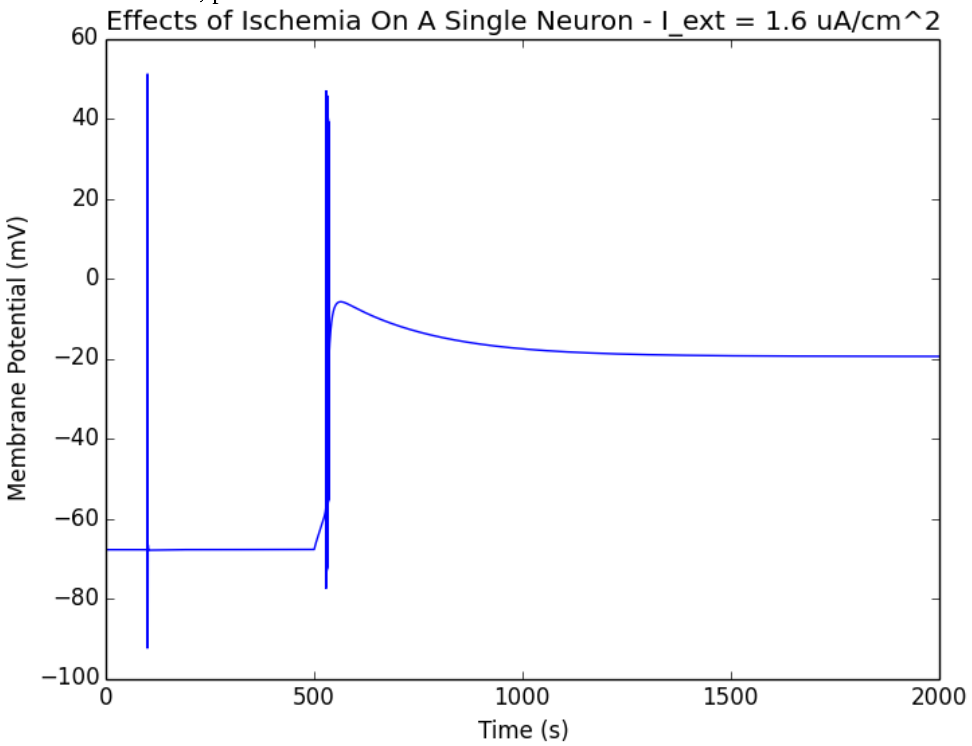


Figure: Slow depolarization of membrane potential following complete OGD. The initial spike is the application of external current (I_ext) of 1.6 µA/cm^2.

The potassium efflux causes the mean membrane potential to increase from around -68 mV to -20 mV. The stability of the membrane potential to -20 mV occurs due to the balancing of the increased potassium channels by the leak channels and thus negates the imbalance in the electrochemical gradient.

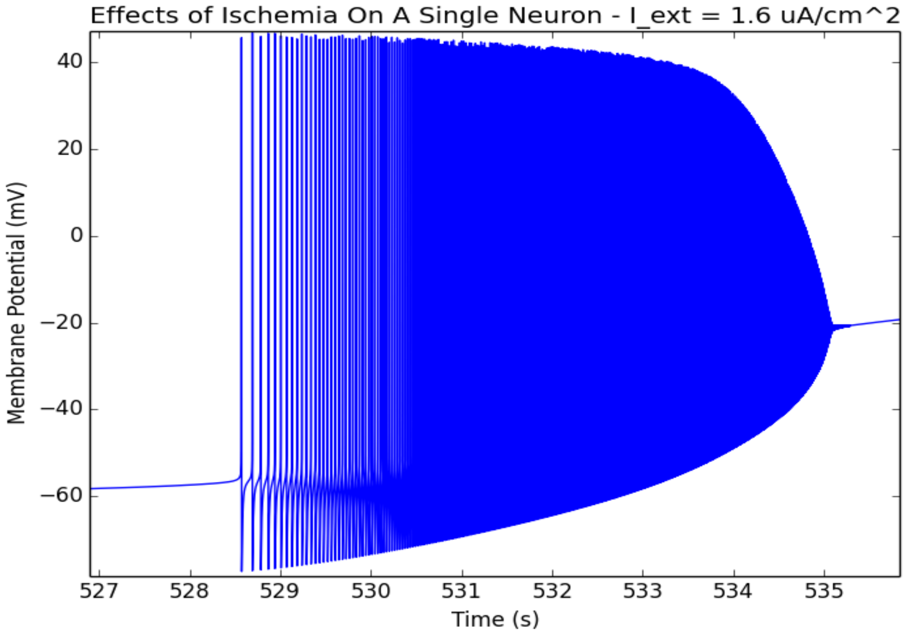
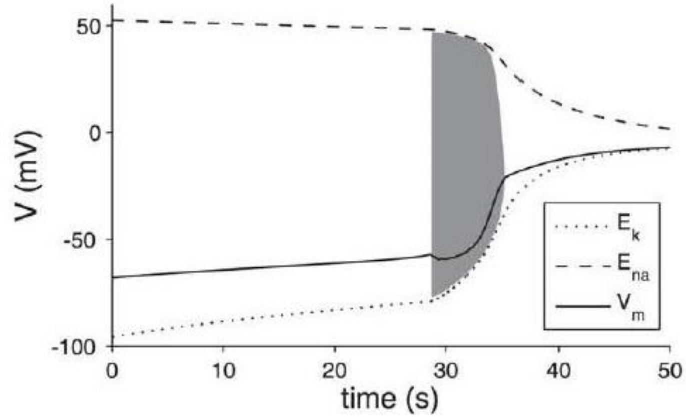


Figure: The sudden spike in membrane potential following severe anoxia occurs due to the positive feedback loop that forms after impairment of the sodium potassium ATPase pump.

This is similar to the EEG observations by van Rijn et al.[1]



In order to simulate the effects of ischemia on larger neuron networks, we attempted to synapse Hodgkin Huxley neurons with dynamic Nernst potentials. We increased the complexity of the ischemic neuronal system as far as the limitations of our coding environment in python would allow. However, due to the time-course of events taking place and the relatively limited computing power at our disposal, we began with a simple two neuron excitatory unidirectional synapse connecting an upstream neuron with a driving current to a downstream neuron without one.

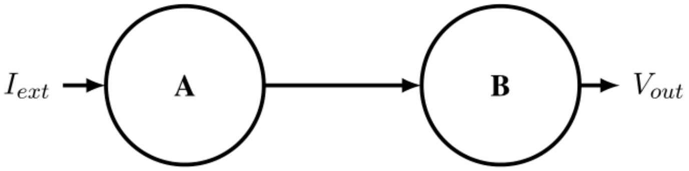


Figure: Simple two-neuron excitatory synapsing motif

With the initial simulations of the neuronal system, we found some interesting behavior in the downstream neuron. As was expected, the excitatory synapse fully functioned in stimulating the downstream neuron during the wave of death in the upstream neuron. For a brief period of time, the downstream neuron, as is seen in the figure below, ceases firing, obviously due to the lack of upstream activity. However, as time progresses, the downstream neuron begins firing continuously, seemingly without any spiking stimulus from the upstream neuron.

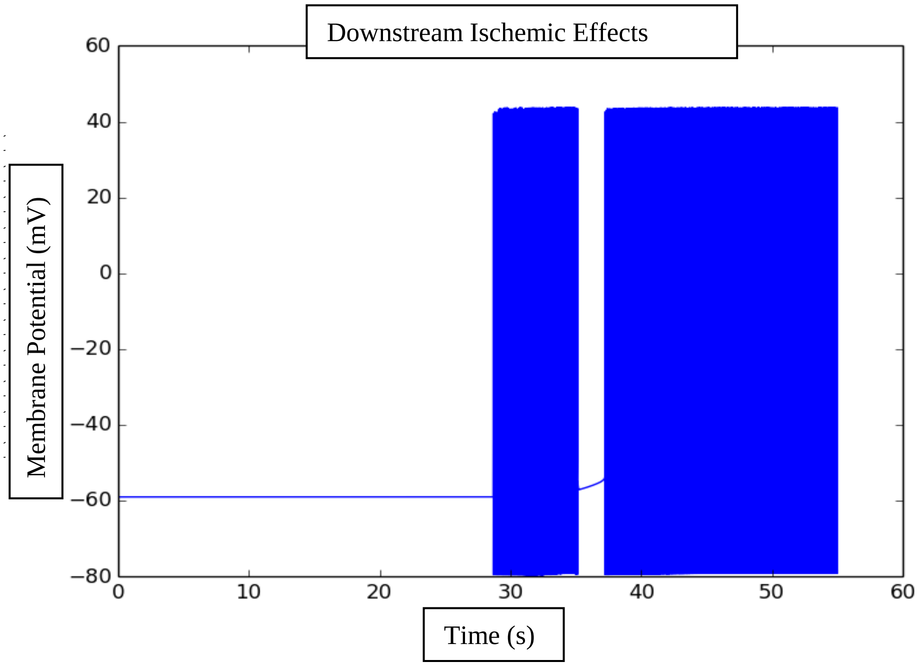


Figure: Neuron B (Downstream) Membrane potential during and immediately after i schemic onset

We determined this problem to be a mathematical one rather than a biological one, residing in the formulation of the differential equations used to update the membrane potential. As the membrane potential equilibrates in the upstream neuron, it most likely achieves a value that is above the reversal potential built into the driving equation for the synapse, causing the synapse to continuously fire, and thus stimulate the downstream neuron at every time point we compute. It is important to note that the ischemic phenomena being considered occur over a time course of 60 seconds in this particular simulation, meaning that 60,000 milliseconds are simulated. Continuous spiking during the ischemic-equilibrated phase of the upstream neuron means that the downstream neuron will only continue to spike at this rate as long as the membrane potential remains elevated, making it both disadvantageous computationally and pointless to further simulate neuronal dynamics after this point. In order to force the simulation to run, specific settings were imposed on the differential equation solver being used to optimize it slightly more for the increased stiffness of the problem.

The final step in the simulations was to determine the vitality of our mathematical models after the ischemic conditions had been placed into effect transiently, and then removed, thus allowing the system to either return to its previous, stable equilibrium, or attain a new resting state. Our model simulated some rather interesting results regarding these two test conditions.

In the most extreme cases of ischemia, our model encountered instabilities when we attempted to restore oxygen and glucose to the cell in the form of reactivation of the corresponding ionic currents. Namely, the dynamic Nernst reversal potentials attempted to calculate based on negative membrane potentials, as given by our differential equations, resulting in a domain error. Though there is no concrete evidence linking this phenomenon to irreversible cell damage, it is interesting to note that our model does not support reversible membrane dynamics after ischemia has persisted for too long.

Therefore, in order to gauge degrees of recovery post-ischemia, we began with a very short ischemic time window, on the order of 30 seconds.

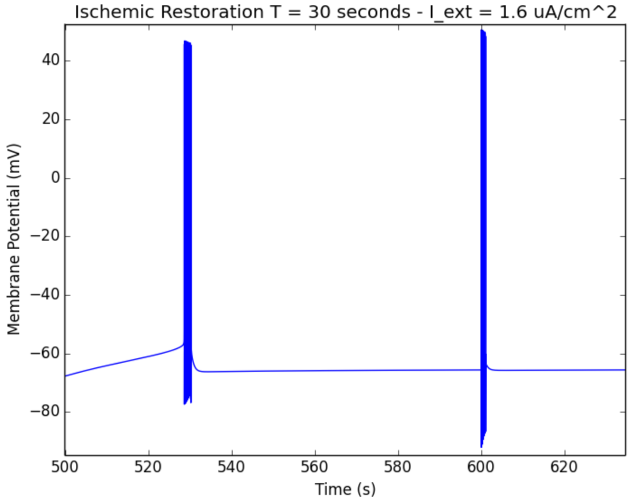


Figure: Restoration of membrane potential after Ischemic onset for a small time duration.

As is evident in the diagram, the ischemic conditions persist for only a short time before the re-introduction of oxygen and glucose allows the membrane to return to its resting state. The first spiking region of the diagram above is the beginning of the wave of death, concurrent with the onset of anoxia. The second spiking region corresponds to a driving current being applied to the neuron. As we can see, the neuron has retained its spiking character and the membrane potential is holding steady at the previously maintained resting potential.

We found, after some experimentation, that the maximum time which our model allowed for partial membrane recovery was after approximately 50 seconds of simulation time. At the 50 second mark, the neuron is still able to recover relatively quickly, over the course of a few seconds, but there is a marked positive drift in the resting membrane potential after it is achieved. The spiking behavior of the neuron is apparently retained, as is evident in the figure below with an applied current at 600 seconds. However, the long term effects of the membrane potential drift need to be further investigated to evaluate whether or not the neuron will have viability issues in the future.

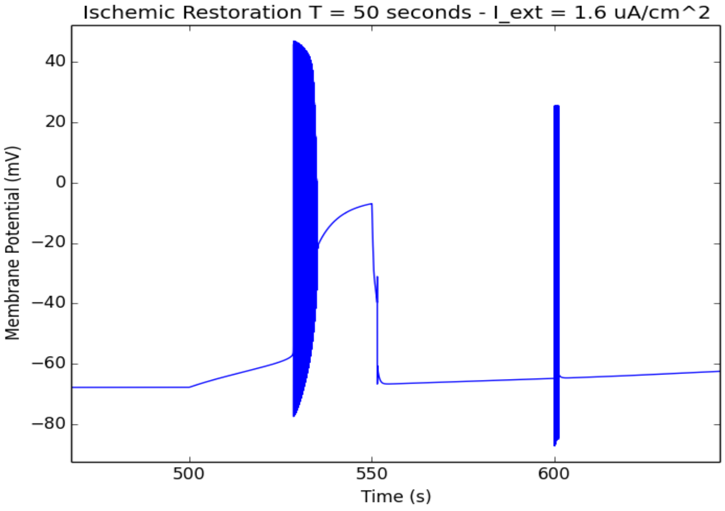


Figure: Maximum Ischemic duration for which spiking recovery was possible

4 Conclusion

Single neuron dynamics in HH neurons reveal that the EEG phenomenon of a slow depolarizing wave in complete anoxic conditions occurs due to the huge efflux of potassium. However, this process is not necessarily a biomarker of irreversible damage and may be reversed upon activation of the sodium-potassium pumps. This can occur, mathematically, during a limited window after the onset of ischemia, after which other biological factors, such as apoptotic signaling and necrosis must be taken into consideration. The network dynamics of ischemic neuron must be optimized, since the current mathematical model does not support simulation on a larger scale. Numerical approximations of the dynamics of the ion concentrations could be used to simulate the wave of death in a much more computationally feasible manner. However, preliminary tests indicate that the wave of death will have a significant effect on the membrane potentials of downstream neurons, and thus cascade through heavily linked neuron networks, likely leading to phenomena such as post-stroke seizures and epilepsy. Nevertheless, in spite of its mathematical instability under certain circumstances, we have successfully implemented a functional Hodgkin Huxley model of ischemia which can be further optimized and applied in subsequent studies.

5 References

[1] van Rijn CM, Krijnen H, Menting-Hermeling S, Coenen AML (2011) *Decapitation in rats: latency to unconsciousness and the ‘wave of death’*. PLos One 6: e16514.

[2] Barreto, E., & Cressman, J. (2011). Ion concentration dynamics as a mechanism for neuronal bursting.

[3] Johanson, C., Duncan, J., Klinge, P., Brinker, T., Stopa, E., & Silverberg, G. (2008). *Multiplicity Of Cerebrospinal Fluid Functions: New Challenges In Health And Disease. Cerebrospinal Fluid Research*, 5(10), 1-32. Retrieved December 20, 2014

[4] Zandt, B., Haken, B., Dijk, J., Michel J. A. M. Van Putten, & Baud, O. (2011). *Neural Dynamics during Anoxia and the “Wave of Death”*. PLoS ONE, E22127-E22127.

6 Code Index

```
#Runmodel.m
from __future__ import division
import numpy as np
from math import exp,log
from scipy.integrate import odeint
import pylab as plt
import sys

class P:
    Tanoxia = 0


def FullModel(y,t):
    if (t > p.Tanoxia[0] and t < p.Tanoxia[1]):
        Apump = p.Ap
        Adiff = p.Ad
        Clconst = False
    else:
        Apump = 1
        Adiff = 1
        Clconst = True

    if ((t > p.Tcurr[0]) and (t<p.Tcurr[1])):   #%inject current when specified
        Iapp = p.Icurr #; %[uA/cm^2]
    elif((t > p.Tcurr[2]) and (t<p.Tcurr[3])):
        Iapp = p.Icurr
    else:
        Iapp = 0
    # Gates
    #  alpha_n = 0.01 * (y[0]+34.0)/( 1.0 - exp(-0.1 * (y[0])+34.0)) #; %[no units]
    alpha_n = 0.01*(y[0]+34.0)/(1.0-exp(-0.1*(y[0]+34.0)))
    beta_n = 0.125 * exp(-(y[0]+44.0)/80.0)
    alpha_m = 0.1 * (y[0]+30.0)/( 1.0 - exp(-0.1 * (y[0]+30.0)) )
    beta_m = 4.0 * exp(-(y[0]+55.0)/18.0)
    alpha_h = 0.07 * exp(-(y[0]+44.0)/20.0)
    beta_h = 1.0/( 1.0 + exp(-0.1 * (y[0]+14.0)) )
    m_inf = alpha_m/(alpha_m + beta_m)
    #% Nernst potentials
    E_k = 26.64 * log(y[3]/y[6])  #;       %[mV]
    E_na = 26.64 * log((y[7]/y[4]))
    E_cl = 26.64*log(y[8]/y[9])
    #% Currents
    Ina = p.g_na*(m_inf**3)*y[2]*(y[0]-E_na) + p.g_naL*(y[0]-E_na) #;  % [mS/cm^2 * mV = uA/cm^2]
    Ik = (p.g_k*y[1]**4)*(y[0]-E_k) + p.g_kL*(y[0]-E_k) #;
    Icl = p.g_clL*(y[0]-E_cl)
    Ipump = Apump*(p.rho/(1.0+exp((25.0-y[4])/3.0)))*(1/(1+exp(5.5-y[3]))) #;   % [mM/s]
    Iglia = Apump*(p.glia/(1.0+exp((18.0-y[3])/2.5))) #;                        % [mM/s]
    Idiffusion = Adiff*p.epsilon*(y[3]-p.kbath)


    dydx = np.zeros(11)
    dydx[0] = (1/p.Cm)*(-Ina -Ik -Icl-0*Ipump+Iapp)
    dydx[1] = p.phi*(alpha_n*(1-y[1])-beta_n*y[1])
    dydx[2] = p.phi*(alpha_h*(1-y[2])-beta_h*y[2])
    dydx[3] = (1/p.tau)*(p.gamma*p.beta*Ik -2.0*p.beta*p.gamma*Ipump -Iglia -Idiffusion)
    dydx[4] = (1/p.tau)*(-p.gamma*Ina -3.0*p.gamma*Ipump)
    dydx[5] = 0
    dydx[6] = -(1/p.tau)*(p.gamma*Ik -2.0*p.gamma*Ipump)
    dydx[7] = (1/p.tau)*(p.gamma*p.beta*Ina +3.0*p.beta*p.gamma*Ipump)
    if Clconst:
        dydx[8] = 0
        dydx[9]= 0
    else:
        dydx[8] = (1/p.tau)*(p.gamma*Icl)
        dydx[9]= -dydx[8]*p.beta
            if(dydx[0] + y[0] > -21 and y[0]<-21):
                        dydx[10] = t-y[10]
            else:
                        dydx[10] = 0
    return dydx

def mainmodel(T,y0):
    p.rcell = 7e-6   #;           % [m], radius of spherical cell
    p.F = 96485.3399  #;         % [C/mol], Faraday constant
    p.gamma = 1e-2*3/p.rcell/p.F     #; % [mM cm^2 /(uA s)] conversion from current to concentration change, gamma = A/(F*V) = 3/(rcell*F)
    p.tau = 1e3  #;           % conversion factor seconds -> ms
    p.beta = 2.0  #;            % ratio intra/extracellular volume;
    p.rho = 1.25/p.gamma   #;          % 1.25 mM/s / (mM cm^2 /(uA s)) = uA/cm^2 , pump current scaling
    p.glia = 200.0/3.0  #;       % mM/s, "pump rate" of [K+]e by glial cells
    p.epsilon = 4.0/3.0  #;      % [1/s] diffusion rate
    p.kbath = 4.0  #;            % [mM], concentration K+ of "bath"
    p.Cm = 1.0   #;               % [uF / cm^2],  membrane capacitance
    p.g_na = 100.0  #;           % [mS / cm^2],  maximum gate conductances
    p.g_naL = 0.0175  #;         % [mS / cm^2],  leak conductance
    p.g_k = 40.0   #;             % [mS / cm^2]
    p.g_kL = 0.05  #;            % [mS / cm^2]
    p.g_clL = 0.05  #;           % [mS / cm^2]
    p.phi = 3.0  #;              % [1/ms],       gate time constant
    tspan = np.arange(0,T+0.1,0.1)
    Sol = odeint(FullModel, y0,tspan,rtol = 1e-3, hmax = 1e3)
    return Sol


y0 = [-67.7966,0.0661,0.9804,3.8280,20.0001,0,138.7929,143.9961,6.0,130.0,0]
#y0p = [-50.0,0.08553,0.96859,7.8,15.5,0,140,144,6,130]
p = P()
p.Tanoxia = np.array([500,550])*1e3  #onset of anoxia
p.Ap = 0
p.Ad = 0
```

```
p.Tcurr = np.array([100,101, 600,601])*1e3 # time between current is injected
p.Icurr = 1.6 # [uA/cm^2]
T = 2000*1e3 # 1000*1e3 #[ms]

Sol = mainmodel(T,y0);

voltage = Sol[:,0]
temptspan = np.arange(0,T+0.1,0.1)

deltime = Sol[:,10]
spkfrq = []
spkfrq.append(0)
for i in range(len(deltime)-1):
        diff = (deltime[i+1]-deltime[i])/1000
        spkfrq.append(diff)


plt.figure()
plt.plot(temptspan*1e-3,voltage)
plt.title('Ischemic Restoration T = 50 seconds - I_ext = 1.6 uA/cm^2')
plt.ylabel('Membrane Potential (mV)')
plt.xlabel('Time (s)')
plt.show()
sys.exit(0)
```