Introduction

Oxygen and glucose deprivation has almost immediate effects on brain function, typically causing symptoms in approximately 5–7 seconds. This dysfunction is also reflected in the electroencephalogram (EEG), generally consisting of an increase in slow wave activity and finally in the cessation of activity. These phenomena are a direct consequence of synaptic failure of pyramidal cells [1], reflecting the high metabolic demand of synaptic transmission [2].

Recent findings in rats, decapitated to study whether this is a humane method of euthanasia in awake animals, indeed showed disappearance of the EEG signal after approximately 15–20 s. After half a minute of electrocerebral silence, however, a slow wave with a duration of approximately 5–15 seconds appeared (Figure 1). It was suggested that this wave might reflect the synchronous death of brain neurons [3] and was therefore named the “Wave of Death”.

Similar experiments were performed by Swaab and Boer in 1972 [4]. The EEG survival time was of the same order as the observations of van Rijn et al [3]; after approximately 7 s the EEG flattened to become iso-electric after 20 s. Recordings did not last longer than that, however, which may explain why the “Wave of Death” was not detected in these experiments.

Van Rijn et al. [3] speculated that the wave might be due to a simultaneous and massive loss of resting membrane potential, caused by the oxygen-glucose deprivation (OGD) following decapitation. Indeed, plenty of (experimental) literature exists showing that hypoxia causes membrane depolarization. Siemkowicz and Hansen [5], for instance, induced complete cerebral ischemia in rats for ten minutes. During and after this period they recorded an EEG and measured the extracellular potential and extracellular ion concentrations. A rapid deflection of the extracellular potential occurred typically 1–2 minutes after the onset of ischemia, accompanied by a sudden rise in extracellular potassium. Unfortunately, EEG activity during the ischemic episode was not described and it is unknown whether a similar wave in the EEG occurred here. Another example is the work of Dzhala et al., who perfused rat brains in vivo with an anoxic-aglycemic solution and measured the transmembrane potential of a pyramidal cell. Approximately eight minutes after the onset of the induced ischemia, they observed a rapid depolarization of the cell membrane [6]. Depolarization is also observed in computational models. For example, Kager et al. modeled neuronal dynamics and ion concentrations and show that an increased concentration of potassium in the neuronal environment can cause fast membrane depolarizations. Depolarization also takes place in their simulations when the ion pump rates are lowered and a neuron is stimulated by injecting current for a few 100 ms [7,8].

In this communication we present a minimal biophysical, single-cell model. Using Hodgkin-Huxley dynamics to describe the voltage-dependent ion channel dynamics, including oxygen/glucose dependent ion pumps, we show that severe oxygen-glucose deprivation results in a sudden depolarization of the membrane voltage. Subsequent modeling of the EEG results in a macroscopic wave, as observed by van Rijn et al. [3]. Finally we discuss that this wave does not reflect irreversible damage and hence not death.

Methods

Biophysical model

A biophysically realistic neuron is modeled using Hodgkin-Huxley dynamics of sodium and potassium channels combined with leak currents. The model includes the dynamics of the extracellular potential and extracellular potassium concentration.
and intracellular ion concentrations, which change significantly when homeostasis cannot be maintained by neurons and glia. Ion pump fluxes are incorporated to model this homeostasis. Our model is based on the equations by Cressman et al. [9–11], who studied the effects of the extracellular ion concentrations in the generation of epileptic seizures.

The model consists of an intracellular and an extracellular compartment separated by a semi-permeable cell membrane. This membrane contains a fast transient sodium channel, a delayed rectifier potassium channel and a leak for sodium, potassium and chloride. The dynamics of the membrane voltage, \( V \), are described with the Hodgkin-Huxley equations:

\[
C \frac{dV}{dt} = -I_{Na}(m_{Na}(V), h, V - E_{Na}) - I_K(n, V - E_K) - I_C(V - E_C) \tag{1}
\]

with \( C \) the membrane capacitance and \( I_{Na}, I_K, I_C \) the total sodium, potassium and chloride currents. The Nernst potential for each ion species is indicated with \( E_x \) and given by \( E_x = \frac{RT}{z_x F} \log \left( \frac{[x]^i}{[x]^e} \right) \), with \( k \) the Boltzmann constant, \( T \) the absolute temperature, \( z_x \) the valency of the ion, \([x]^i\) and \([x]^e\) the intra- and extracellular concentrations and \( x = Na, K, Cl \). The fraction of activated sodium channels, \( m_{Na}(V)^3 \), is due to its fast dynamics assumed to depend instantaneously on the membrane voltage. \( h \) is the fraction of inactivated sodium channels and is a variable in our model. \( n \) is the fraction of activated potassium channels and is also a variable. The calcium gated current from the Cressman model is not implemented, because it does not qualitatively alter the behavior of interest here. We write for the total sodium, potassium and chloride currents

\[
I_{Na} = g_{Na} m_{Na}(V)^3 h(t) [V - E_{Na}(t)] + g_{Na,L} [V - E_{Na}(t)]

I_K = g_K n(t)^4 [V - E_K(t)] + g_{K,L} [V - E_{K,L}(t)]

I_C = g_{Cl} [V - E_{Cl}(t)],
\]

respectively. The maximum ion conductances for the gated currents are denoted with \( g_x \) and for the leak currents with \( g_{Cl} \).

The gating variables \( m_{Na}(V) \), \( n \) and \( h \) are modeled as [11]:

\[
m_{Na}(V) = \frac{z_{Na}(V)}{z_{Na}(V) + \beta_{Na}(V)}

z_{Na}(V) = (V + 30 mV)/[1 - \exp(-(V + 30 mV)/10 mV)] \cdot 10 mV

\beta_{Na}(V) = 4 \exp(-(V + 55 mV)/(18 mV))

\frac{d}{dt}(\phi[z_{Na}(V)(1 - q) - \beta_{Na}(V)q]) = n, h \tag{3}
\]

where \( \phi \) is the time constant of the channels. When the ion concentrations, on which the Nernst potentials depend, are assumed to be constant, equation sets 1 to 3 can be used to model the dynamical behavior of a single neuron.

In order to calculate changes in ion concentrations in the model, equations are added that integrate the ion fluxes into and out of the two compartments. During physiological conditions, the concentrations are given by [11]:

\[
d[Na]_i \frac{dt}{dt} = \frac{A}{VF} (-I_{Na} - 3 I_p) \quad d[Na]_a \frac{dt}{dt} = - \frac{\beta A}{VF} (-I_{Na} - 3 I_p) \tag{4}
\]

\[
d[K]_i \frac{dt}{dt} = \frac{A}{VF} (-I_K - 2 I_p) \quad d[K]_a \frac{dt}{dt} = - \frac{\beta A}{VF} (-I_K - 2 I_p) \quad -I_K - I_d \tag{5}
\]

\[
d[Cl]_i \frac{dt}{dt} = 0 \quad d[Cl]_a \frac{dt}{dt} = 0,
\]

with \( A \) and \( V \) respectively the surface area and volume of the cell, \( F \) the Faraday constant and \( \beta \) the ratio of the intra- and extracellular volumes. \( I_p \) denotes a sodium-potassium pump current (in \( mA/cm^2 \)) which depends sigmoidally on the intracellular sodium concentration and the extracellular potassium concentration. The total amount of sodium is preserved in this model, but the extracellular potassium can be buffered by glial cells \( I_d \) and can diffuse from and into the blood \( I_d \). Furthermore, the chloride concentrations are assumed to remain constant under normal conditions, without specifying the mechanism for this. The approximation that the efflux of potassium equals the influx of sodium made by Cressman et al. in order to reduce the number of variables is not made here.

The pump, glial and diffusion currents are modeled as [11]:

\[
I_p = \frac{\rho_p}{1 + \exp(25mM - [Na]_i)/(3mM)} \times \frac{\rho_p}{1 + \exp(5.5mM - [K]_i)/(1mM)} \nonumber 
\]

\[
I_d = \alpha ([K]_i - k_x),
\]

Here \( \rho_p \) scales the pump rate, \( G \) the glial buffering rate, \( \alpha \) is the time constant of diffusion and \( k_x \) the concentration of potassium.
in the blood. Note that $I_p$ and $I_d$ do not have the dimension of current, but that of rate of change of concentration (mM/s).

**Numerical implementation**

Equation sets 1 to 5 completely describe our model. The resting state of this system is calculated, with the parameters shown in table 1. The equations were solved with a solver for stiff ordinary differential equations (ode23 routine, Matlab, the Mathworks). The simulation code is available from ModelDB [12], accession number 139266. Table 2 shows the results of this calculation, which are used as starting point for the simulation of oxygen and glucose deprivation. It was verified that the model behaves as expected under normal circumstances: in rest the membrane potential and the sodium and potassium concentrations are in the physiological range. Furthermore the neuron responds with a single action potential when a short current pulse is applied and spikes periodically when a current of 1.5mA/cm² or more is injected.

**Simulating oxygen and glucose deprivation**

To simulate the anoxia and aglycemia, we set both the pump current and the uptake of K⁺ ions by the glial cells to zero as well as diffusion of K⁺ to the blood. Furthermore the chloride concentrations are no longer assumed to stay constant. This changes the equations for the concentration dynamics, Eqns 4, into:

$$\frac{d[x]}{dt} = -\frac{\beta A}{V_zF} I_x, \quad \frac{d[x]}{dt} = -\frac{A}{V_zF} I_x, \quad \text{for } x=K,Na,Cl$$

(6)

**Results**

In the case of a normally functioning neuronal unit, which maintains homeostasis, the model reaches a steady state with a membrane potential of 20 mV after about ten minutes (not shown).

### Table 1. Overview of the parameters used in the simulations.

| variable | value | units | description |
|----------|-------|-------|-------------|
| $C_m$    | 1.0   | µF/cm² | specific membrane capacitance |
| $g_{na}$ | 100   | mS/cm² | sodium channel conductance |
| $g_{leak}$ | 0.0175 | mS/cm² | sodium leak conductance |
| $g_{pk}$ | 40    | mS/cm² | potassium channel conductance |
| $g_{kl}$ | 0.05  | mS/cm² | potassium leak conductance |
| $g_{cl}$ | 0.05  | mS/cm² | chlorine leak conductance |
| $\delta$ | 3     | ms⁻¹   | time constant of gating variables |
| $A/VF$  | 0.044 | mM/(ml/min) | conversion factor current to concentration |
| $\beta$ | 2.0   |       | ratio intra-/extracellular volume |
| $\rho_p$ | 28.1 | µA/cm² | NaK-Pump rate |
| $G$     | 66    | mM/s   | glial buffering rate of K⁺ |
| $\tau$  | 1.3   | s⁻¹    | diffusion rate |
| $k_w$   | 4.0   | mM     | concentration K⁺ in blood |
| $\tau$  | 310   | K      | absolute temperature |

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In order to compare the simulated single cell behavior with the EEG observed by van Rijn et al. [3], we proceed as follows. The contribution of a single cell to the (raw) EEG is roughly proportional to its membrane potential [13]. Modeling the EEG realistically usually requires a large scale simulation with many neurons, because the behavior of a cell depends heavily on its interaction with other neurons. The present situation provides an exception, however, because synaptic transmission has stopped and neurons receive no direct input. As a result, their dynamics can be accurately described with a single cell model; the EEG of an ensemble of cells can be calculated by simply summing the contributions of individual neurons. Assuming that many neurons behave approximately the same as the modeled neuron, but with some small shift in time, the resulting raw EEG is proportional to...
the mean membrane potential (Figure 3, dashed line). For simplicity, a flat distribution of 300 ms wide was chosen, but varying the shape and width of this distribution hardly changes the resulting EEG. High-pass filtering the resulting potential with a cut-off at 0.1 Hz replicates the filter characteristics of the filter used by van Rijn et al. [3]. This results in the solid curve shown in Figure 3, similar to the reported “Wave of Death” (cf Figure 1 used by van Rijn et al. [3]). This results in the solid curve shown in Figure 3, similar to the reported “Wave of Death” (cf Figure 1).

**Discussion**

Dynamic phenomena that occur during hypoxia and the way they are reflected in the EEG are only partially understood. Measurements of extreme cases showing clear features in the EEG present an opportunity to gain insight in the relation with the underlying physiology. Such an extreme case is decapitation, in which the supply of energy to the entire brain is halted almost instantaneously. This causes the EEG to become flat after several seconds, but also results in a large amplitude wave approximately a minute after decapitation. Van Rijn et al. suggest that this wave “ultimately reflects brain death” [3], but also state that further research on the physiology of brain function during this process is needed.

We modeled the membrane voltage dynamics of a single neuron with a sodium and a potassium channel and leak currents, together with the corresponding changes in the intra- and extracellular ion concentrations. This model can explain the physiological origin of the wave. When a sodium-potassium pump, glial buffering and diffusion of potassium are incorporated to model homeostasis, the model shows regular behavior and has a resting state where all variables obtain values in their physiological ranges. After shutting down the energy supply, the membrane initially depolarizes slowly with a slope of approximately 0.7 mV/s, until it reaches the excitation threshold, around ~58 mV. Now spiking starts, resulting in an increase in the potassium current with a concomitant reduction in the potassium Nernst potential and membrane voltage. Positive feedback between the increasing firing rate and potassium efflux causes a sudden depolarization of the membrane voltage (30 mV in 2 seconds), resulting in the membrane depolarization curve, displayed as a dashed line in Figure 3. In combination with a high-pass filter, the simulated membrane voltage results in a wave in the EEG as observed by van Rijn et al. (Figure 3, solid line). This behavior was also observed in the in vivo measurements in rats by Siemkowicz and Hansen [5], who also measured a rapid depolarization accompanied with an increase of extracellular potassium, typically 1–2 minutes after the onset of ischemia. While modeling the effects of decapitation, an instantaneous cessation of the sodium-potassium pump, glial buffering and diffusion of potassium to the blood was assumed. The last assumption is very reasonable, because arterial pressure vanishes after decapitation, larger vessels are drained and blood flow through the capillaries will stop. The (remaining) blood volume is relatively small and the ion concentrations in the blood will therefore quickly equilibrate with the tissue. However, a complete stop of all active ion transport will not take place directly after decapitation. Some reserves of metabolic substrates and ATP are still left in the tissue. In human brain tissue for example, these reserves can support a maximum of one minute of normal metabolism [14], but less if no oxygen is available. Such effects do not disqualify the general behavior of the model, as they will only result in a delay in the onset of depolarization, in line with the observations by van Rijn et al. Siemkowicz and Hansen [5] hypothesized that the transition from a slow to a fast rise of extracellular potassium and the corresponding depolarization is the result of depletion of these energy reserves; they hypothesized that the pumps are initially still partially fueled by anaerobic glycolysis until the glucose reserve is depleted and the ion pumps.

![Figure 2. Membrane dynamics during oxygen-glucose deprivation](image)

In the left panel the membrane dynamics are shown that occur after the onset of OGD (solid line). The dashed and dotted lines show the progressive loss of ion gradients. When after a gradual rise the membrane potential reaches the excitation threshold, this subsequently results in spiking of the membrane voltage according to Eqs 1 and 2 (gray region, not resolved). The black line shows the average membrane potential during the spiking (averaged over 300 ms). After approximately 7 seconds of oscillations, the cell comes to rest again, with a resulting $V_m \approx -20$ mV. The middle panel shows a close up of the start of spiking activity, the right panel shows the instantaneous firing rate.

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observed speed of propagation in these slices is too slow to account glutamate or by interaction through gap junctions [8]. The depolarization is hypothesized to be caused by diffusion of speed of approximately 0.1 mm/s [17]. The propagation of depression and hypoxia respectively. 1–3 minutes after the onset of injecting KCl or halting the oxygen supply, to simulate spreading who induced depolarization in hippocampal slices, by either Such a synchronization has indeed been measured by Aitken et al., single pyramidal neuron are of the order of pA, much too small to

**References**

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