Non-trivial dynamics in a model of glial membrane voltage driven by open potassium pores

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Abstract - Despite the molecular evidence that close to linear steady-state current-voltage relationship in mammalian astrocytes reflects a total current resulting from more than one differently regulated $K^+$ conductances, detailed ODE models of membrane voltage $V_m$ incorporating multiple conductances are still lacking. Repeated experimental results of deregulated expressions of major $K^+$ channels in glia, Kir4.1, in models of neurodegenerative disease, as well as their altered rectification when assembling heteromeric Kir4.1/Kir5.1 channels have motivated us to attempt a detailed model incorporating the weaker potassium K2P-TREK1 current, in addition to Kir4.1, and study the stability of the resting state $V_r$. The main question is whether with a deregulated Kir conductivity the nominal resting state $V_r$ remains stable, and the cell retains a potassium electrode behavior with $V_m$ following $E_K$. The minimal 2-dimensional model near $V_r$ showed that certain alterations of Kir4.1 current may result in multistability of $V_m$ if the model incorporates the typically observed $K^+$ currents - Kir, K2P, and non-specific potassium leak. More specifically, a decrease or loss of outward Kir4.1 conductance (turning the channels into inwardly rectifying) introduces instability of $V_r$, near $E_K$. That happens through robustly observed fold bifurcation giving birth to a much more depolarized second, stable resting state $V_m^{dr} > -10 \text{ mV}$. Realistic time series were used to perturb the membrane model, from recordings at the glial membrane during electrographic seizures. Simulations of the perturbed system by constant currents through gap-junctions and transient seizure-like discharges as local field potentials led to depolarization of the astrocyte and switching of $V_m$ between the two stable states, in a down state – up state manner. If the prolonged depolarizations near $V_m^{dr}$ prove experimentally plausible, such catastrophic instability would impact all aspects of the glial function, from metabolic support to membrane transport and practically all neuromodulatory roles assigned to glia.

1. Introduction

Membrane voltage control in glia - The temporal dynamics of membrane voltage ($V_m$) of glial cells, the non-excitable neural cells, is a difficult subject to treat due to their structure-function complexity, and the experimental limitations to precisely measure $V_m$ by classical electrophysiological methods. The classical, more than 50 years old view of the glial membrane as a passive potassium electrode (Kuffler, Nicholls, & Orkand, 1966) reflected observations that $K^+$ conductances in astrocytes accounting for the major part of ion conductivity near the resting voltage, are of Ohmic nature. It is important to note that those early observations, lacking molecular specificity, have come from studies with a rather narrow experimental focus on the glial role in ion homeostasis, i.e., in the removal of the excess extracellular $K^+$ following sustained neuronal firing. Intensive molecular studies in the last twenty years seriously challenged the view of purely passive electrochemical response capability of glia, upon evidence that their membrane voltage $V_m$ is transiently perturbed by different electrogenic pathways through transporters and ionotropic receptors (Kettenmann & Steinhaeuser, 2005), expressed with certain variations by both neurons and glia. It is now known that glial glutamate and GABA transporters, and even more intriguingly ionotropic glutamate and GABA receptors in some glia cell types (Bedner, Jabs, & Steinhaeuser, 2019), do alter glial $V_m$, (Kettenmann, Backus, & Schachner, 1984) and therefore introduce a form of feedback control of their respective neuromodulatory functions. Observations that receptor activation leads to a transient suspension of major potassium conductance (Schröder, Seifert, Hüttmann, Hinterkeuser, & Steinhaeuser, 2002), likely to be coupled with one of several mechanisms of $Na^+$ intake or extrusion (Felix, Delekate, Petzold, & Rose, 2020), further requires a careful distinction of how each of those effects qualitatively and quantitatively affects the glial $V_m$.

Therefore, with the glial $V_m$being both, a control variable (controller) of different neuromodulatory loops, as well as itself being a target of neuromodulatory perturbations, the dynamical stability of...
glial resting membrane voltage $V_r$ in response to various perturbations is still a central question in quantitative whole-cell modeling of glia. Such a dual effect of $V_m$ as a variable, further qualifies the glial membrane as a sensor and transducer of $K^+$ volume transmission as a signal, which perspective has been pushed by pioneers in the field (Ransom, 2000) but further demands explanatory biophysical models.

The typical, transient perturbations of glial $V_m$ comes either as: (i) transient variations of local field potential which directly polarize the membrane, (ii) varying trans-junctional voltage at the gap-junction connections of heavily interconnected astrocytic cells, or (iii) as transiently altered $[K^+]_o$. Different biological control roles of glial $V_m$ makes the stability of $V_r$ critical for a wide range of cellular functions from glucose transport and neurotransmitter recycling, via responses to mechanical and acidic stress, to the regulation of excitation-inhibition balance in local circuits. Prospective dynamical modeling studies would try to distinguish the specific, causal responses of glial $V_m$ to a specific form of neuromodulation, from the permanent stochastic electrochemical or other perturbations of $V_m$ near the $V_r$.

**Variations of $V_m$ in a steady and perturbed glial membrane** - Experimental studies of astrocytes in situ, the most abundant glial cell subtype, find that their $V_m$ in nominal conditions fluctuates within a narrow range (Henneberger & Rusakov, 2012). From several millivolts, in response to nominal fluctuations of the local field, up to $10 \div 20 \text{ mV}$ more depolarized from $V_r$ in cases of seizures, other strong synchronous discharges in the neighboring neurons and spreading depolarizations accompanied by high, transiently elevated $[K^+]_o$ (Traynelis & Dingledine, 1988), (Ballanyi, Grafe, & ten Bruggencate, 1987), or (Somjen, 2004) for review. Even though accumulated extracellular $K^+$ depolarizes glia, it is not clear how fast, and how closely glial $V_m$ follows the perturbed $E_K$ when both active and passive transport mechanisms switch in, and what is in turn the sign of the driving force $\Delta V_m = V_m - E_K$ that direct the leaky currents. Typically, the reversal potential of the isolated macroscopic Kir and K2P currents is close, but slightly more positive to the Nernstian reversal potential of potassium, $E_K$, and closer to the cell resting voltage $V_r$ which is typically between $-80 \text{ mV}$ to $-70 \text{ mV}$ in glial cells, in situ. A relevant dynamical model of $V_m$ would thus require incorporating the *minimal*, yet detailed description of the major glial ion conductances active near $V_r$, over several timescales from milliseconds to minutes, under assumption it does not incorporate $[K^+]_o$ dynamics, i.e., only $E_K$ remains a changeable parameter.

Figure 1 shows the steady-state current-voltage (I-V) recordings of the total $K^+$ current, as well as the isolated Kir4.1 and K2P-TREK1 currents in our data, obtained under whole-cell voltage-clamp protocol in $N = 9$ freshly isolated astrocytes from mouse hippocampus, bathed in slightly elevated extracellular $[K^+]_o = 5 \text{ mM}$ (Seifert et al., 2009). In steady-state conditions under a physiological $K^+$ concentration gradient, the barium $Ba^{2+}$- sensitive weakly rectified Kir4.1 current (circles) dominates the total current profile (solid line). Even though much weaker in resting conditions, the K2P current (triangles) has different kinetics and electrochemical regulation, which requires to have it incorporated into any quantitation of $K^+$ conductivity in astroglia, either experimental or theoretical.

**Figure 1 - Potassium current profile in isolated astrocytes** – Kir4.1 current isolated as $Ba^{2+}$-sensitive, by application of 0.1mM BaCl (blue circles/line, n=9) reverses at $V_{rev} \approx 75.35 \text{ mV}$, compared to the total $K^+$ current, black solid line (data from (Seifert et al., 2009)). Of the remaining $K^+$ conductance, K2P current (max. 0.23 nA at +30mV), was isolated as quinine-sensitive, plotted in green triangles/line. The transcript analysis of the K2P channels suggested a prevalence of K2P2.1 TREK1 isoform (Seifert et al., 2009). The sum of Kir4.1 and K2P2.1 current accounts for 82% of the total current at -120mV, and 80% at +30mV. Recordings from 9 cells, with a mean effective patch capacitance of 20 pF, have been normalized, averaged, and rescaled in the measured current range.
We described the I-V curves of the dominant $K^+$ background currents in isolated astrocytes from mouse hippocampus (Seifert et al., 2009). The isolated $K^+$ currents operate close to, and therefore define the resting membrane potential $V_m$ in the proposed minimal ordinary differential equation (ODE) model of $V_m$ dynamics. Apart of $K^+$ conductances, no other major conductances have been verified in astrocytes in the voltage range around $V_r$, assuming no osmolar stress which triggers non-negligible $Cl^-$ transients. Co-expressed Kir and K2P channels account for the major fraction of potassium channels in almost all glial cells in the brain (Steinhäuser, 2013), cardiac fibroblasts (Zuo, 2017), as well as in various cell types in renal epithelia (Hebert, 2005), (Welling, 2016). With over forty different channel isoforms in total, the co-occurrence of Kir and K2P channels in these, as well as in other tissues (Sepúlveda, 2015) is pervasive, resulting in different $K^+$ channel mix and therefore different resulting I-V curves and kinetic properties of the currents.

Parametric analysis of the model reproducing some of the observed biological (de)regulation of those conductances suggests that astrocytic $V_m$ could be prone to multistability in case of deregulation of the major weakly-rectified Kir4.1 current (WR-Kir). Numerical simulations of the model perturbed using realistic recordings from glia in-situ (Traynellis & Dingledine, 1988), illustrated the bistability of the otherwise stable resting state. Although a mix of only $K^+$ conductances may give rise to two membrane resting states, already observed experimentally in cardiomyocytes (Zuo, 2017), and described by a simple, generic minimal 2-dimensional conductance-based model (Izhikevich, 2007), so far it has not been demonstrated in the glial membrane, or simulated numerically using a glia-specific model.

2. **Model of astroglial whole-cell $V_m$ dynamics** – Detailed electrophysiological studies of glial$^1$ conductances (Seifert et al., 2009) are rare, and a widely accepted minimal dynamical model of $V_m$ in astrocytes is therefore lacking. Further to the experimental complexity mentioned above, depending on the model system, sometimes a specific regulation of these conductances by other physiological stimuli require quantitation.

In what follows we outline the description of experimentally measured steady-state I-V relationships of Kir4.1 and K2P-TREK1 conductances. The nonexistence of a voltage-sensing domain - a structure present in other gated $K^+$ channels, suggests their rectification properties come from permeability regulation by physiological blocking ions, or by other Coulombic interactions on a pore level (Gonzalez et al., 2012) resulting in voltage activation. The modeling approaches to either Kir or K2P currents do not follow the Hodgkin-Huxley approach for gated channels but reflect their known biophysics and biology as open pores and the recordings of their steady-state I-V curves. The proposed models could be applied as-are to a different mix of those channels in different cells or tissues. In the rest of the text, for the cell-specific aspects, without loss of generality, we will refer to rodent astrocytes from the hippocampus, as our model system (Seifert et al., 2009).

2.1 **Steady-state I-V model of weakly-rectified Kir current** - Since the early quantitative studies, the conductivity of Kir channels under changing $[K^+]_o$, (Hagiwara & Takahashi, 1974) indicate that Kir channels display voltage dependence on the electrochemical driving force $\Delta V_m = V_m - E_K$, rather than solely on the net membrane voltage $V_m$. This implies that suggested models should: (i) allow for variable $[K^+]_o$, and (ii) describe qualitative changes on different timescales, relevant not only for the millisecond $V_m$ dynamics, but for the slower $[K^+]_o$ variations from hundreds of milliseconds to seconds and minutes, as well.

Our central modeling proposition is a detailed description of the I-V relationship of the weakly-rectifying potassium Kir current (WR-Kir), rather than straightforwardly assigning the Hagiwara model of a strongly inwardly-rectifying channel in egg cells (Hagiwara & Takahashi, 1974). The Hagiwara model is usually chosen under the assumption that $V_m$ is very tightly regulated round the negative resting potential of $V_r \approx -80mV$, which is oversimplification because astrocytic $V_m$ is exposed to perturbations that significantly depolarize the cell into a $V_m$ range where the curves of

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$^1$ In the rest of the text, we will interchangeably use the terms glia, astroglia or astrocytes for the population of the astrocytic cells. Where reference is made to other glial cell types, those are named specifically.
inwardly and weakly rectified Kir current markedly differ. To fill this gap requires describing quantitatively the outward conductivity of WR-Kir channels, which represent a comparable fraction to the inward component, Fig. 1 (circles).

The key modeling assumption is that the steady-state WR-Kir current, Fig. 1 (circles), could be dissected into two additive components: a) inwardly rectifying Kir component \( I_{K_{ir}} \), and b) outwardly rectifying, or residual Kir component \( I_{res} \), so that for the total isolated \( B_{K_{ir}}^2 \)-sensitive Kir4.1 current we can write:

\[
I_{K_{ir}} = I_{inw} + I_{res} \quad \text{(nA)}.
\] (1)

The two unidirectional fluxes result from a different manifestation of a voltage-dependent block of the pore by polyvalent cation which in turn result in different outward and inward permeation. Cases are known where point mutations have dominantly impacted unidirectional permeation in open pores (Wible, Tagliatela, Ficker, & Brown, 1994), (Kurata, Rapedius, Kleinman, Baukrowitz, & Nichols, 2010) We describe this using a short-pore model of \( K^+ \) pore, formulated using a simplified description of the permeation based on one-dimensional reaction coordinate. It is generic for all \( K^+ \) open pores, where the cytoplasmic domain of the channel is ignored as not critical for describing the permeation, Fig. A1, Appendix 1. For a detailed description of the short-pore model and further biophysical arguments on the legitimacy of modeling two separate, additive fluxes in the Kir short-pore, see Appendix-1.

**Inwardly rectified WR-Kir flux** results from a negative driving force, \( \Delta V_m < 0 \), where the blocking ions, \( M \) \( g^+ \) or other polyvalent cations are kept within the water cavity (Fig. A1.B) of the short-pore by Coulombic forces of the negative residues of transmembrane helices (Fig. A1.A). Such ion-crowding of permeant and blocking ions (Fig. A1.B) results in an inward pseudo gating of the WR-Kir pore producing an equilibrium probability of open, inwardly conducting pore described by Boltzmann term, as originally introduced by the Hagiwara model (Hagiwara & Takahashi, 1974):

\[
I_{inw} = g_{K_{ir}-inw}(V_m - E_{K_{ir}}) \quad \text{(nA)}
\] (2)

\[
g_{K_{ir}-inw}(\Delta V_m) = \frac{A \cdot \delta_{s-inw} \sqrt{[K]_o}}{1 + \exp(-z_{inw}(\Delta V_m - \Delta V_{12})/\nu_s)} \quad \text{(\muS)}
\] (3)

\[
\Delta V_m = (V_m - E_{K_{ir}}), \quad \Delta V_{12-inw} = (V_{12-inw} - E_{K_{ir}}) \quad \text{(mV)},
\]

where \( \delta_{s-inw} \) represents the maximal value of the \( g_{K_{ir}-inw} \) obtained as a slope of the linear segment in the recorded I/V curve negative of \( E_K \); \( V_{12-inw} \) is the voltage at half-maximal conductance, \( z_{inw} \) represents the effective charge valence of permeant ions in the short-pore and \( E_{K_{ir}} = -76 \text{ mV} \) is the reversal voltage of the isolated Kir4.1 current. Figure 2A, black curve, shows a fitting sigmoid curve (3) to numerically differentiated Kir4.1 data, from Fig. 1 (blue curve, under \( [K^+]_o = 5\text{mM} \), for \( V_m \) more negative than \( E_{K_{ir}} \). Such numerically obtained series physically represents the conductance, whereas the values more negative to -110mV (crosses) are discarded due to slope deviation (Fig.1) originating from the voltage-dependent pore block by external \( Na^+ \) (Ohmori, 1978), (Kubo, Baldwin, Jan, & Jan, 1993). The fitting gave \( \delta_{s-inw} \sqrt{[K]_o} = 20.6 \text{ nS}, V_{12-inw} = -53.5 \text{ mV} \) and \( z_{inw} = 1.638 \). The effective charge \( z_{inw} \), was in accordance with published charge estimates for the voltage dependence of block by small ions, for a summary see (B. Hille, 2001). The slope factor \( \nu_s \equiv RT/F = 25.7 \text{ mV}, \) corresponding to room temperature of \( T = 298.13 \text{ K} \) is kept constant throughout the text.

**Outwardly rectified Kir4.1 flux** results from outward pseudo gating for a more positive driving force \( V_m > -60 \text{ mV} \) (Fig. A1.C), where outwardly directed driving force pushes partly or fully dehydrated blocking ion towards the entry of the selectivity filter (SF), see Appendix 1. Such electrostatic, flickering block modulates ion association to the pore and the outward permeation.
To obtain numerical series for the residual, outward Kir4.1 current $I_{\text{res}}$ (4) from the recordings, we subtracted the portion described by the Hagiwara model in the whole $V_m$ range, equations (2),(3), fitted near $V_r$, Fig.2B, blue line, from the total Kir current, to obtain the red trace/triangles in Fig.2B.

$$I_{\text{res}} = I_{K_{\text{ir}}} - I_{\text{inv}} \quad (nA).$$

Non-constant field Goldman-Hodgkin-Katz (GHK) model of WR-Kir outward current - Meyer B. Jackson reminds us in his seminal monograph (Jackson, 2006) of several concepts always potentially applicable to an open channel when there are indications of specific pseudo-gating or macroscopic current rectification, like in potassium Kir or K2P pores. In other words, without a voltage gate as a structural determinant for gating, other features like physiological block by cytoplasmic cations may produce charge movement within the membrane or alter the Coulombic and/or structural forces defining open-pore permeability within the single global state of the channel.

We arrive at the proposed model of the residual current using the following assumptions:

- **GHK formalism without the constant-field** describes the outward conductivity, using (5) as a general form of GHK current equation before introducing the constant-field assumption (Jackson, 2006), chapter 13.13. Instead, a simple, quadratic potential energy profile of an elastic force $U(x) = U_{\text{max}} - w(x - x_T)^2$ introduces a parabolic energy barrier in the energy profile of an open Kir pore, Fig.3A, with the voltage drop over the pore added as linear, $V_m(x) = x\Delta V_m$. The $U_{\text{max}}$ potential represents the peak of the association barrier to the permeant $K^+$ ion at the entry of hydrophobic SF in RT units. The barrier is centered at transition state $x_T$, while the positive $w$ is the force constant of the elastic restoration force of Coulombic nature. For the charge in molar equivalents and the concentrations in $[mM]$, we will describe the outward current using:

$$I_{\text{res}} = zFD \frac{[K^+]_r - [K^+]_o e^{-zFV_m/RT}}{\int_a^b e^{U(x) - zFV_m(x)}/RT \, dx} \quad (nA).$$

We can extend the interval $(a, b)$ to $(−\infty, +\infty)$ because the energy profile falls sharply outside the short pore. Depending on the description of $U_{\text{max}}$ dependence on $V_m$, which is a critical parameter, the location of $x_T$ could change, resulting in an asymmetrical shape of the barrier, Fig. 3A, dashed line, compared to the symmetrical case in Fig. 3B corresponding to $\lambda_1 \neq \lambda_2$.

- **Partial voltage-dependent pore occlusion by cytoplasmic cations activates the pore in the outward direction** – In this model voltage-driven localization of polyvalent cytoplasmic cations in the cavity produces a partial, flickering pore occlusion, Fig. 3A, which increases the Coulombic repulsion on the permeant ion associated with the SF. Such partial blocking serves as a plausible modulatory mechanism, with the pore remaining in a single open conformation. Therefore, both, the pseudo-gating on pore-level and the macroscopic current rectification are attributed to the voltage dependence of the block. This defines a voltage-dependent probability of an activated pore with an effective, equivalent charge $z_{eq} > 1$, reflecting the valence of the blocking ion ($Mg^{2+}$ or positively charged polyamines), not...
necessarily implicating multi-ion pore. We illustrate this blocking configuration along the reaction coordinate of the short-pore model on the sketch in Figure A1.C, as a displacement of the blocking $Mg^{2+}$ ion from S6 (center of the cavity) towards the S5 ion coordination site (SF entrance). To formulate this modulatory blocking effect as a probability of outward activation in a non-gated channel, we use the Boltzmann equation in a classical way (Jackson, 2006), chapter 1.12:

$$p_{\text{out}} = \frac{1}{1 + e^{-z_\beta(V_m-V_{12-\text{out}})/\nu_s}}. \quad (6)$$

Since both, the Hagiwara model of Kir pores, as well as more recent permeation studies on K2P (Schewe et al., 2016), suggested electro-chemical gating in $K^+$ pores, with effective driving force $\Delta V_m = V_m - E_K$, applying it within Boltzmann equation cancels out the reversal potential $E_K$ in (6), as it also appears in the half-activation voltage $\Delta V_{12-\text{out}} = V_{12-\text{out}} - E_K$. The slope factor $\nu_s$ is the same defined within the model for inward flux.

- **Quadratic dependence of barrier peak $U_{\text{max}}$ on voltage (Marcus form)** - We are adding nonlinear dependence (7) to introduce a simple form of Coulombic contribution of the voltage-dependent block in $U_{\text{max}}$ according to (Jackson, 2006), see chapters 7.6 and 7.8:

$$U_{\text{max}}(V_m) = G_0 \left( \frac{\lambda - \frac{z_\beta \delta (V_m - E_K)}{4\lambda \nu_s G_0}}{\nu_s} \right)^2 \quad (RT \ units), \quad (7)$$

which can describe the saturation of outward flux for very positive applied voltages, Fig.4, to be discussed later. Equation (7) is based on the Marcus chemical kinetics theory describing the charge transfer in chemical bonding, using reaction rate models (Marcus, 1964), which has meanwhile gained a wider acceptance in treating quasi-equilibrium changes in proteins, for review see (Matyushov, 2015). We stress that we use here qualitative features of the simplified one-dimensional reaction coordinate in the Marcus theory, to describe macroscopic properties, like the saturation in the voltage dependence of block in experimental measurements of whole-cell currents, rather than to construct a reaction rate model which is typically done for predicting permeation rates (Peters, 2015).

Figure 3B shows qualitatively the geometry of the Marcus energy landscape and $U_{\text{max}}(V_m)$ over the simplified, one-dimensional reaction coordinate. The peak of the barrier $U_{\text{max}}$ at the intersection of the two parabolic wells, defines the transition state $x_T$, separating the left energy well where the permeant ion dwells before the outwardly directed field drives it to the SF, and the right well where the ion is associated to the pore. The critical parameter influencing $U_{\text{max}}$ voltage dependence is the fraction of the energy profile $\delta$, which falls under

![Figure 3](image-url)
top (dashed line) in (A). Here, compared to (A), the reaction coordinate spreads over the whole short-pore, and 
\[ x_T = 1/2 \] places the peak of the barrier at the entry of SF. (C) and (D) peak behavior of \( U_{\text{max}} \) (7) and non-
constant GHK permeability (9) at \( V_C = -23.2 \text{ mV} \) which will produce maximal outward current \( I_{\text{res}} \). The peak of
\( P_{\text{GHK}} \) in (D) suggests that the Coulombic nature of the M\( g^+ \) block facilitating the outward permeation produces a
maximal \( P_{\text{GHK}} \) at \( V_C \) after what it gradually turns into full pore occlusion for more positive \( V_m \).

the linear voltage drop, reducing it to \( \delta \Delta V_m \). Different positions of the transition state \( x_T \) for \( \delta \Delta V_m =
0 \) have been intentionally selected in Fig. 3A (\( x_T = l_{SP}/3 \)), and 3B (\( x_T = l_{SP}/2 \)) to illustrate the shift of
\( x_T \). Figure 3C illustrates the quadratic profile of \( U_{\text{max}}(V_m) \) with the minimum attained at a critical
voltage \( V_C = -23.2 \text{ mV} \), at which we observe the maximal permeability \( P_{\text{GHK}} = P_K = 7.63 \times 10^{-8}
\text{ cm/s}. Fig. 3D, of the same order of magnitude as the ranges in the early studies of rectification in
potassium channels, see (Lu, 2004) for a review. The zero RT minimum at \( U_{\text{max}}(V_C) \), Fig. 3C,
should be interpreted as a zero of the voltage-dependent potential energy profile at \( x_T \) - where the
voltage term in (7) rises the potential energy of the associated ion leveling it with \( \varepsilon_0 \), the height of the
entry barrier representing the image force of hydrophobic SF. Introducing nonlinear \( U_{\text{max}}(V_m) \) is
significant for capturing the outward WR-Kir current saturation with voltage (Meeks & Mennerick,
2007), (Edvinsson, 2011), (Gonzalez et al., 2012) which is not achieved with a constant or linearly
dependent \( U_{\text{max}} \).

Under the above assumptions, by solving the Gaussian integral in the nominator of (5) and

simplifying the solution by setting a fixed position \( x_T = 1/2 \) (Jackson, 2006), for the outward residual component \( I_{\text{res}} \) of isolated Kir4.1 current we obtain:

\[
I_{\text{res}} = p_{\text{out}} zF P_{\text{GHK}} \left( [K^+]_1 e^{\frac{zV_m}{2e\varepsilon}} - [K^+]_0 e^{-\frac{zV_m}{2e\varepsilon}} \right) \quad \text{(nA).} \tag{8}
\]

\( x_T = 1/2 \) places the transition state and \( U_{\text{max}} \) at the entry of the SF where the blocking ion should
be localized for positive \( \Delta V_m \), which is close to 1/2 of the length of the short pore with the cavity
approximately 10Å in diameter, and the SF length typically approximated to 12 \( \div \) 15Å, depending on
the structure-function assumptions. The simplification removes \( x_T(\Delta V_m) \) shift which, for illustration,
for \( \Delta V_m = v_s, l = 1/4 \), with \( z_B = 1.6 \) and \( \varepsilon_0 = 6.6 \text{ RT} \) obtained by fitting (8) to the data (4), shifts \( x_T \)
from 0.5 to 0.56.

Having departed from the classical Nernst-Planck description of the open pore to an alternative form
of GHK equation for the macroscopic \( I_{\text{res}} \) in (5) and (8), extended with modulation of permeability by
voltage-dependent block, we implicitly define voltage-dependent permeability (9) (Jackson, 2006).

\[
P_{\text{GHK}}(V_m) = \frac{W}{\pi RT} D e^{-\frac{U_{\text{max}}(V_m)}{RT}} = P_K e^{-\frac{U_{\text{max}}(V_m)}{RT}} \quad \text{(cm/s).} \tag{9}
\]

Here \( w \) is the same force factor from the harmonic potential \( U(x) \), and \( D \) is the classical, constant
diffusion coefficient, both absorbed within the constant, voltage-independent \( K^+ \) permeability \( P_K \)
multiplied by a factor of Arrhenius activation form with voltage-dependent barrier \( U_{\text{max}}(V_m) \). For
other studies where explicit, nonlinear dependence of GHK permeability has been used, mostly in
neuronal calcium channels, see (Borg-Graham, 1999).

\[^2 \delta \text{ represents a fractional, dimensionless, “electrical” length of the pore or permeation coordinate } 0 \leq \delta \leq l_{SP} \equiv 1.\]
For the final form of the Kir current model to fit the isolated Kir4.1 current recordings, we get:

\[
I_{K_{ir}} = I_{inw} + I_{res} \quad \text{(nA)}
\]

\[
I_{inw} = g_{Kir}(V_m - E_K) = \frac{A g_{K_{ir}} \sqrt{[K^+]_o}}{1 + e^{-z_{inw}(V_m-V_{12})/v_z}} (V_m - E_K) \quad \text{(nA)}
\]

\[
I_{res} = p_{out} zF P_K e^{-u_{max}/RT} \left( [K^+]_i e^{zV_m/v_z} - [K^+]_o e^{zV_m/v_z} \right) \quad \text{(nA)}
\]

Fitting \( I_{res} \) model (10) with \( U_{\text{max}} \) given by (7) to the residual outward current data obtained by (4), using nonlinear least-squares error, gives the I-V graph in Figure 4A. The peak behavior in (7) produces the declining slope in the rise of \( I_{res} \) and a peak behavior in a wider positive, non-physiological \( V_m \) range for glia, Fig.4B, as already reported in a study of a whole rodent hippocampus (Meeks & Mennerick, 2007), with the curve shifted to more negative voltages.

All the parameter values, fixed and obtained by fitting are listed in Table 1. The table values of \( I_{res} \) fitting parameters: \( P_K, V_{12-out}, G_0, z_B, \) and \( z \), to be later used in the parameter analysis of the model, were obtained as averages of ten curve fitting attempts with one of them varied within \( \pm 10\% \) of the initial value in each run while the other four were recalculated by the NLSQ curve fitting. The blocking ion charge valence was bounded within \( 1.5 \leq z_B \leq 2.0 \). The goodness of fit \( R^2 \geq 0.98 \) was achieved in all attempts.

2.2 Steady-state I-V model of K2P-TREK1 current – Studies isolating the weaker astrocytic currents after isolating Kir currents are rare, due to experimental issues with leaky astrocytic membrane, and therefore K2P currents are rarely quantified (Seifert et al., 2009), (Zhou M., 2009). Figure 5A shows pharmacologically isolated K2P current, using quinine, at \([K^+]_o = 5 \text{ mM}\) (black circles), as well as at drastically elevated external potassium at \([K^+]_o = 50 \text{ mM}\) (blue dots) in our data from (Seifert et al., 2009). Both I-V recordings were fitted (solid lines) with the standard GHK current equation (Chen, 2014), even though the permeation studies suggest multi-ion occupancy of the pore and some form of pseudo gating (Schewe et al., 2016). Adding a small \( Na^+ \) permeability (Chen, 2014), \( P_{Na}/P_K = 0.06 \), within nominally non-conductive range (below \(-50 \text{ mV}\)) improves the fit at \( 5 \text{ mM} \), solid purple line. That effect of \( Na^+ \) on passive \( K^+ \) conductivity in glia is known (Ransom & Goldring, 1973), but we didn’t use the correction within the numerical simulations due to a lack of recordings to verify \( P_{Na}/P_K \) dependence itself on \([K^+]_o \) for notably elevated levels above \( 5 \text{ mM}\).

The K2P currents in this study (Seifert et al., 2009) originate primarily from the K2P-TREK1 sub-population of K2P channels. TREK1 channels are polymodal transducers of different cellular stimuli (Honore, 2007), (Enyedi & Czirjak, 2010), like mechanical pressure or pH shifts that transduce into changes of \( V_m \), so that any distinction between steady-state and activated state of these and most of others K2P channels requires careful consideration, both experimental and biophysical. Working with steady-state recordings from astrocytes isolated in a bath assures that obtained I-V curves represent cells in steady conditions, non-transducing an osmolar stress or pH variations, which is implicitly assumed in a baseline model.
Figure 5 - Steady-state I-V relationships and activation kinetics of K2P-TREK1 currents in astrocytes – (A) Steady-state I-V characteristics of K2P-TREK1 currents fitted with the Goldman-Hodgkin-Katz current equation, for \([K^+]_o = 5\text{mM}\) (black circles/line) and 50mM (blue circles/line). The purple curve shows the fit corrected with a small \(Na^+\) permeability being added \((P_{Na}/P_{K} = 0.06,\) see text). (B) and (C) Approximation of the activation kinetics \(n_{K2P}(V)\) of K2P current for \([K^+]_o = 5\text{mM}\) and 50mM respectively, obtained as \((\bar{g}_{slope} / \bar{g}_{max})^+(1/k)\). 

Apart from the outward voltage activation (verified also in a symmetrical \(K^+\) concentrations), K2P channels show also electrochemical activation component coming from multi-ion occupancy of the pore by the permeant \(K^+\) ions (Schewe et al., 2016). The following two dynamical properties in voltage-dependent activation of K2P-TREK1 current are critical for demonstrating nontrivial behavior in the dynamic model of \(V_m\):

- **Activation kinetics** - At both concentrations, the whole-cell currents showed activation kinetics with \(\tau_{K2P} \approx 3\text{ ms}\), constant over the whole \(V_m\) range. While at 5mM, above –50 mV we could fit a Boltzmann sigmoidal, voltage-dependent activation, Fig. 5B black dots, at drastically elevated, \([K^+]_o = 50\text{mM}\) there is no visible voltage dependence, Fig. 5C, described with a constant average activation of \(\langle n_{K2P} \rangle = 0.66\).

- **Gating mode change** – At elevated external \(K^+\), Fig. 5A, the dominating electrochemical activation (Schewe et al., 2016) is changing the I-V curve, resembling more that of the leaky K2P-TWIK channels with almost linear dependence, satisfying the GHK current equation and reversing close to the Nernst potential.

Along with these observations we model the astrocytic K2P-TREK1 currents using the GHK current model (Chen, 2014), extended with electrochemically dependent activation \(n_{K2P}\) – coupling the voltage-dependent activation and electrochemical driving force into a mechanism named ion-flux gating (Schewe et al., 2016):

\[
I_{K2P} = n_{K2P}^k I_{K2P-GHK} = n_{K2P}^k P_{K2P} \frac{F^2 z_{K2P}^2}{RT} \left(V_m - V_{o_{fS}}\right) \frac{\left([K^+]_o - [K^+]_o \exp\left(-z_{K2P}(V_m - V_{o_{fS}})/v_s\right)\right)}{\left(1 - \exp\left(-z_{K2P}(V_m - V_{o_{fS}})/v_s\right)\right)} \text{nA.}
\]

(11)

Allowing for variations of \(K^+\) concentrations we cannot keep the intrinsic permeability \(P_{K2P}\) constant (Eisenman, 1983), contrary to the classical assumptions based on the solubility-diffusion theory (B. Hille, 2001), (Johnston, 1995). To add a simple \([K^+]_o\) dependence in \(P_{K2P}\) we interpolated the fitted values for \(P_{K2P}\) at 5mM and 50mM with an increasing and slowly saturating \(K^+\) dependence (12a).

The GHK fit at 5mM suggested \(P_{K2P}^0 = 1.24 \times 10^{-8}\text{cm/s}\) for the baseline permeability, within the order of magnitude with those reported in the early studies of electro-diffusion in \(K^+\) channels, (Hodgkin & Horowicz, 1959). Fitting of the sigmoid in Fig.5B to \((\bar{g}_{slope} / \bar{g}_{max})^{(1/k)}\) graph obtained
by differentiation for both concentrations was done with \( k = 2 \), following the average ion occupancy of the SF with 2.2 \( e_0 \) elementary charge units across different K2P channel subtypes, estimated in (Schewe et al., 2016). Small voltage offset \( V_{ofs} \) not exceeding 5 \( mV \) was needed to stabilize numerically the nonlinear LSQ fitting of (11). A same \( z_{K2P} = 1 \) value was used in both \( K^+ \) concentrations.

\[
[K^+]_{o}^b \quad \text{the nominal, baseline external concentration in (12a) was kept at 2.5 mM. To correct the } n_{K2P} \text{ activation for variable concentrations two interventions are needed: (i) decreasing trend of the maximal activation with } [K^+]_{o} \text{ (12b), where the } [K^+]_{o}/[K^+]_{i} \text{ term successfully captures the trend, and (ii) shifting the } V_{12-K2P} \text{ (12c) and the whole } n_{K2P} \text{ sigmoid to the left (into non-physiological hyperpolarized range) so that the plateau of } n_{K2P} = 0.66 \text{ covers the most of physiological } V_m \text{ range – practically removing voltage-dependent activation, Fig. 5C. More recordings on different } [K^+]_{o} \text{ are needed to verify whether the suggested corrections in (12b) and (12c) have more general biophysical value. The shift of Nernstian nature in (12c) required a scaling factor } S = 1.7 \text{ to get the plateau-like in Fig. 5C. Similar proportionality of } V_{12} \text{ shift was observed in human K2P-TREK1 channels expressed in } Xenopus \text{ oocytes (Schewe et al., 2016).}
\]

\[
P_{K2P} = P_{K2P}^b (1 + 0.85 \log_{10} ([K^+]_{o}/[K^+]_{i}^b)) \quad (\text{cm/s}), \quad (12a)
\]

\[
n_{K2P}(V,[K^+]_{o}) = \frac{1 - [K^+]_{o}/[K^+]_{i}}{(1 + \exp(-z_{K2P} F (V - V_{12-K2P})/RT))}, \quad (12b)
\]

\[
V_{12-K2P}([K^+]_{o}) = V_{12-K2P}^b - S \ln ([K^+]_{o}/[K^+]_{i}^b) \frac{RT}{z_{K2P} F} \quad (\text{mV}). \quad (12c)
\]

Equation (11) fuses (i) the modified voltage-dependent and \([K^+]_{o}^b\)-corrected \( n_{K2P} \) activation (12b) describing an arbitrary K2P channel population, and (ii) the nonlinear Goldman-Hodgkin-Katz electro-diffusion describing the macroscopic steady-state current of the whole, voltage-clamped cell. The GHK current model extended with activation kinetics has already been used in descriptions of neuronal voltage-gated calcium channels (Destexhe, 2000).

### 2.3 Full dynamical model of glial \( V_m \) – The dynamics of the astrocytic membrane voltage \( V_m \) is described by the differential equations (13), where to Kirchhoff’s current law a single kinetics equation has been coupled, that of K2P channel activation \( n \).

\[
C_m dV_m/dt = -(I_{Kr} + I_{K2P} + I_{leak}) + I_{ext} \quad (\text{nA})
\]

\[
dn/dt = (n_{K2P}(V_m) - n)/\tau_{K2P}. \quad (13)
\]

An unspecific, potassium Ohmic leak current is added as \( I_{leak} = g_{leak} (V_m - E_K) \) with the conductance not exceeding 15% of the chord conductance of total glial \( K^+ \) current in the I-V plot, proportional to the current that remains after isolating Kir and K2P currents, Fig 1.

The external inputs, to resemble external currents to an astrocyte in situ, are represented as:

\[
I_{ext} = I_{gjc} + I_{tfp} \quad (\text{nA})
\]

\[
I_{gjc} \sim \sum_i g^i_{leak} (V^i_m - V_m) = \text{const.} \quad (\text{nA}) \quad i = 1 \ldots N_{\text{neighbors}} \quad (14)
\]

\[
I_{tfp}(t) = g_{tfp} V_{tfp}(t) \quad (\text{nA}),
\]

where \( I_{gjc} \) brings in the contribution from all neighboring astrocytes connected via Ohmic gap junction connections (GJCs) and will be varied as a parameter, while \( I_{tfp}(t) \) represent the transient, time-dependent variable depolarization of the membrane coming from the changing local field.
potential immediate to the astrocytic membrane, resulting from major prolonged neuronal spiking episodes.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Description</th>
<th>Value, default</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T$</td>
<td>$K$</td>
<td>Temperature</td>
<td>298</td>
<td>Room temperature, 25°C in (Seifert et al., 2009)</td>
</tr>
<tr>
<td>$F$</td>
<td>C/mole</td>
<td>Faraday constant</td>
<td>96485</td>
<td></td>
</tr>
<tr>
<td>$R$</td>
<td>$j/mole$·$K$</td>
<td>Universal gas constant</td>
<td>8.314</td>
<td></td>
</tr>
<tr>
<td>$v_k$</td>
<td>mV</td>
<td>Slope factor, $RT/F$</td>
<td>25.7</td>
<td>at $T = 298°K$, constant in all models and simulations</td>
</tr>
<tr>
<td>$[K^+]_{\text{nom}}^{\text{in}}$</td>
<td>mM</td>
<td>Baseline $[K^+]_i$ in extracellular space</td>
<td>2.5 – 5</td>
<td>5mM used in I-V fitting, varied within param. analysis</td>
</tr>
<tr>
<td>$[K^+]_i$</td>
<td>mM</td>
<td>Astrocytic $[K^+]_i$</td>
<td>130</td>
<td></td>
</tr>
<tr>
<td>$C_m$</td>
<td>pF</td>
<td>Astrocytic membrane capacitance</td>
<td>20</td>
<td>Mean effective capacitance of the patch, whole-cell</td>
</tr>
<tr>
<td>$g_{S\text{-inv}}$</td>
<td>$\mu S$</td>
<td>Max. Kir inward slope conductance</td>
<td>0.00917</td>
<td>Hagiwara model of Kir current, Eq. (3)</td>
</tr>
<tr>
<td>$A$</td>
<td>$1/mM^{1/2}$</td>
<td>Dim. constant, correcting for $\sqrt{[K^+]_{\text{inom}}}$</td>
<td>1</td>
<td>Dimensionality correction</td>
</tr>
<tr>
<td>$V_{12\text{-nom}}$</td>
<td>mV</td>
<td>Half-activation voltage $V_{12}$ at $[K^+]_{\text{nom}}^{\text{in}}$</td>
<td>-53.5</td>
<td>Hagiwara model of Kir current, Eq. (3)</td>
</tr>
<tr>
<td>$z_{\text{low}}$</td>
<td></td>
<td>Effective charge valence, $I_{\text{kir}}$</td>
<td>1.638</td>
<td>Hagiwara model of Kir current, Eq. (3)</td>
</tr>
<tr>
<td>$z$</td>
<td></td>
<td>Unitary valence of permeant $K^+$ ions</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>$E_{\text{rev-Kir}}$</td>
<td>mV</td>
<td>Rev. voltage of isolated Kir4.1 current</td>
<td>-76</td>
<td>Exp. measured rev. Voltage, constant</td>
</tr>
<tr>
<td>$p_K$</td>
<td>cm/s</td>
<td>Constant permeability of $K^+$ in pores</td>
<td>7.63e-08</td>
<td>$K^+$ permeability in classical Nernst-Planck formalism</td>
</tr>
<tr>
<td>$V_{12\text{-out}}$</td>
<td>mV</td>
<td>Half-activation voltage of $I_{\text{res}}$</td>
<td>-51.4</td>
<td>Boltzmann term in $p_\phi$, Eq. (6)</td>
</tr>
<tr>
<td>$Z_B$</td>
<td></td>
<td>Effective charge valence of the blocker</td>
<td>1.6</td>
<td>$Mg^{2+}$ or polyamine blocking ion</td>
</tr>
<tr>
<td>$G_0$</td>
<td>RT units</td>
<td>Voltage-independent entry barrier</td>
<td>6.6</td>
<td>Barrier height at transition state, Marcus term, Eq. (7)</td>
</tr>
<tr>
<td>$l_{SP}$</td>
<td></td>
<td>Normalized, unitary length of the pore</td>
<td>1</td>
<td>Short pore model, Fig. A1</td>
</tr>
<tr>
<td>$x_T$</td>
<td></td>
<td>Fractional distance of transition state</td>
<td>1/2</td>
<td>Position relative to the beginning of the pore</td>
</tr>
<tr>
<td>$\lambda$</td>
<td></td>
<td>Half-width of the energy wells</td>
<td>1/4</td>
<td>Marcus term, Eq. (7) and Fig. 3</td>
</tr>
<tr>
<td>$\delta$</td>
<td></td>
<td>Fractional, electrical length of the pore</td>
<td>1/2</td>
<td>Fraction of $l_{SP}$ over which $V_{12}$ drops, Marcus term, Eq. (7)</td>
</tr>
<tr>
<td>$V_{12\text{-K2P}}$</td>
<td>mV</td>
<td>Half activation voltage $V_{12}$ at $[K^+]_{\text{nom}}^{\text{in}}$</td>
<td>-20.5</td>
<td>$I_{K2P}$ model, $n_{K2P}$ activation, Eq. (12c)</td>
</tr>
<tr>
<td>$k$</td>
<td></td>
<td>k-th power in K2P activation kinetics</td>
<td>2</td>
<td>Hodgkin-Huxley formalism, Eq. (11)</td>
</tr>
<tr>
<td>$S$</td>
<td></td>
<td>Scaling parameter in $n_{K2P}$ activation</td>
<td>1.7</td>
<td>Adjusts the shift of $n_{K2P}$ with $[K^+]_i$, Eq. (12c)</td>
</tr>
<tr>
<td>$z_{K2P}$</td>
<td></td>
<td>Charge valence for K2P pores, fixed</td>
<td>1.0</td>
<td>$I_{K2P}$ model, Eq. (11) and $n_{K2P}$ activation Eq. (12b)</td>
</tr>
<tr>
<td>$r_{K2P}$</td>
<td>ms</td>
<td>Activation time of K2P-TREK pore</td>
<td>3.0</td>
<td>From exp. data (Seifert et al., 2009), Eq. (12b)</td>
</tr>
<tr>
<td>$p_{K2P}^0$</td>
<td>cm/s</td>
<td>K2P channel permeability, basal</td>
<td>1.24e-08</td>
<td>$I_{K2P}$ model, GHK description, Eq. (12a),</td>
</tr>
<tr>
<td>$g_{\text{leak}}$</td>
<td>$\mu S$</td>
<td>Glial leak conductance, Ohmic</td>
<td>0.001-0.002</td>
<td>Non-specific leak, glial voltage dynamics, Eq. (13)</td>
</tr>
<tr>
<td>$I_{\text{ext}}$</td>
<td>nA</td>
<td>External current to the astrocyte</td>
<td>0.2 - 0.4</td>
<td>Glial voltage dynamics, Eq. (13)</td>
</tr>
<tr>
<td>$g_{\text{fP}}$</td>
<td>$\mu S$</td>
<td>Transfer conductance in resp. to $V_{fP}$</td>
<td>0.01-0.016</td>
<td>Adjusted to the $I_{\text{ext}}$ ranges in bifurcation analysis</td>
</tr>
</tbody>
</table>

Table 1 – Parameter values used in the fitting of current models, parametric analysis, and simulations of the full system (13).

It has been already shown that equation (13) represents a minimal model where only $K^+$ conductances, without any $Na^+$ or $Ca^{2+}$ contributions could produce more complex dynamical behavior of $V_m$ than a stable fixed point $V_c$ (Izhikevich, 2007).

Table 1. contains all the parameter values and/or value ranges used in the dynamical analysis of the model and the numerical simulations of transiently perturbed behavior.
3. Results

3.1 Parametric analysis of the $V_m$ dynamics

When translating the nature and the intensity of the perturbations in real cells in different experimental preparations into changes of model parameters, we will refer to (a) static alterations of the astrocytic $K^+$ conductances to changes manifesting in the steady-state I-V relationships, and to (b) dynamical perturbations of $V_m$ in (13) for response to transient changes in the local field potential, depolarizations through the gap-junctions, or response to transient shifts of $\left[K^+\right]_o$.

Of not many studies differentially isolating glial currents (mostly focusing on Kir), we looked for those where the changes would alter the monotonic I-V dependence of the total astrocytic current, Fig. 1. For a summary of changes, and the source references see Table 2 in Appendix 2. Sole focus on Kir currents with K2P current not being isolated has prevented those studies to report the impact of altered Kir current on the I-V curve of total current. The alterations of Kir currents summarized in Table 2 suggest that the model should consider asymmetrical changes of Kir conductivity in different $V_m$ ranges, either as a hallmark of a pathology or different functional channel expressions like in the case of heteromeric Kir4.1/Kir5.1 channels. Biophysical specificity of the Kir4.1/Kir5.1 channel is that it turns weakly rectifying Kir4.1 into a strong inward rectifier (Marmolejo-Murillo et al., 2021), numerically equivalent to almost fully attenuating the outward $I_{res}$ current.

Figure 6A illustrates the trend of change in the steady-state I-V curve of the total $K^+$ current in different scenarios of Kir deregulation. A typical effect is a loss of monotonicity positive to $+60\text{mV}$, where both Kir4.1 and K2P currents coexist producing a non-monotonous N-shaped I-V curve. In Figure 6 we sketched this effect by different attenuations of Kir inward and/or outward component in our model, based on the observations in different disease models, Table 2. Included also, the yellow curve, is the combined effect of loss of outward Kir conductance in parallel with drastically increased $\left[K^+\right]_o$ like in cases of seizures and spreading depolarizations. To produce the effects in Fig. 6A we were (a) decreasing $g_{s-inw}$ for the inward range of WR-Kir current in Hagiwara model (3), and (b) doing the same using a multiplier in the GHK model (8) for the outward range, due to a complex form of the conductance in $I_{res}$ model. The K2P current was kept in the nominal range and shape.

![Image](image_url)

Figure 6 – Loss of monotonic I-V curve via alterations of Kir4.1 currents in disease models. (A) Alterations in Kir conductivity without changes in K2P conductance produce non-monotonic I-V curves. The yellow curve illustrates the parallel effect of a drastic increase in external $K^+$, while all others are obtained by amplifying or attenuating the modeled Kir currents at $\left[K^+\right]_o = 5\text{mM}$ based on the nature of deregulation in Table 2. The labels in the legend relates each curve to the corresponding study in Table 2. (B) Example of nullcline analysis in $(V_m, n)$ plane. Increasing the constant $I_{ext}$ changes (into an N-shape) and shifts the V-nullcline introducing a saddle fixed point $V_c$ (red triangle), and markedly more positive stable node or focus $V_{dr}$, via the fold bifurcation. The dotted, dashed, and the full blue line show the changing shape of the V-nullcline with $I_{ext}$.

---

3 Even though the channel expressions change in time, those happen on time scales of minutes, hours or longer, which warrants keeping the corresponding parameters constant on timescales of milliseconds or seconds.
Since the typical dynamic perturbations, same as with neurons transiently depolarize the glial membrane by \( I_{\text{glj}} \) or \( I_{\text{frp}}(t) \) currents, the principal bifurcation parameter was a constant in time \( I_{\text{ext}} \). As a second bifurcation parameter, we varied \( g_{s-\text{inw}} \) and \( [K^+]_o \) with attenuation of \( I_{\text{res}} \) as a static perturbation of the model, so that all scenarios of impact on Kir conductivity could be implemented.

In the 2-dimensional system (13), using nullclines let us detect the trends of change in the phase portrait. As expected and previously illustrated in models of neurons very similar to (13) (Izhikevich, 2007), chapter 5, in addition to the single equilibrium state \( V_r \) - a stable node for \( V_m \) slightly more positive to \( E_K \), the N-shaped I-V curve introduces multiple steady states via the fold bifurcation, as we vary \( I_{\text{ext}} \), Fig. 6B. The fold bifurcation is generically present in 2-d models like (13) with N-shaped nonlinearity in the voltage\(^4\) equation and robustly observed within wide parameter ranges. We keep referring in the rest of the text to the nominal stable, resting state as \( V_r \), and to the more depolarized, again stable steady-state introduced by the fold bifurcation as \( V_{dr} \).

**Figure 7** – Fold bifurcations in \((V_m,n_{K2P})\) dynamics are generic when the perturbed model displays an N-shaped I-V characteristic. (A) Fold bifurcation diagram of (13) for \( g_{s-\text{inw}} = 0.00917 \mu S \), with \( I_{\text{res}} = 0 \). (B) A cusp curve in \((I_{\text{ext}},g_{s-\text{inw}})\) plane obtained by running 2-parameter continuation from the fold point at \( I_{\text{ext}} = 0.2306 \) in (A). The coordinates of the corresponding stable steady states are \((V_r = -77.86 \text{ mV}, n_{K2P} = 0.0964)\), and of the saddle-node close to \((V_m = -9.734 \text{ mV}, n_{K2P} = 0.6)\), with \( g_{s-\text{inw}} \) kept at the same value as in (A). (C) The red dashed line shows a typical path through the parameter plane corresponding to a membrane with deregulated Kir conductance perturbed by a net depolarizing current \( I_{\text{ext}} \). The region bounded by the cusp curve separates the two **monostable** parameter domains, the down-state domain, corresponding to the true nominal resting state \( V_r \) (of node type), and that of the up-state, corresponding to a **single** depolarized steady state \( V_{dr} \), of focus/spiral type. For parameter values inside the transition region, within the cusp, the model displays two stable nodes separated by an unstable saddle point.

Figure 7A and 7B show the fold and cusp bifurcation diagrams for changing \( I_{\text{ext}} \) and \( g_{s-\text{inw}} \), with the outward \( I_{\text{res}} \) attenuated. All continuations were done using the AUTO package (Doedel et al., 1997) as implemented within XPPAUT (Ermentrout, 2002). Examples in Fig.7A and 7B were computed with \( I_{\text{res}} = 0 \), though qualitatively the same behavior is present with \( I_{\text{res}} \) between zero and 15% of its intensity. All other model parameters were kept at the values given in Table 1, used in the fitting of steady-state I-V characteristics, with \( [K^+]_o = 2.5 \text{ mM} \) and the glial leak \( g_{\text{leak}} = 0.0013 \mu S \). At \( I_{\text{ext}} = 0.2306 \text{ nA} \), in Fig. 7A the bifurcation introduces a saddle-node at \( V_{dr} = -9.734 \text{ mV} \), notably depolarized to the nominal \( V_r = -77.86 \text{ mV} \), which further splits into a saddle and stable node at \( V_{dr} \) as \( I_{\text{ext}} \) further increases. The cusp curve Fig. 7B fusing fold bifurcation behavior of two parameters divides the \((I_{\text{ext}},g_{s-\text{inw}})\) plane in three regions: (I) the **down-state** represented by \( V_r \), (II) the **up-state** represented by the depolarized resting voltage \( V_{dr} \), and (III) the bistability region where \( V_m \) transits towards either of the equilibria, qualifying the model for switching behavior. We borrowed these terms from models of neuronal excitability since both neurons and glia share the same feature - the true resting state near \( E_K \) which gets excited by a shift of \( V_m \) to more positive values. Equations (13) represent the first minimal mathematical model of the glial membrane near \( V_r \), based on whole-cell

---

\(^4\) We refer to the first ODE in (13) as voltage equation because it describes the \( dV_m/dt \) derivative, even though as a physical law it is the Kirchhoff’s current equation.
recordings, suggesting the existence of a stable, depolarized up-state in glial $V_m$ dynamics, within a wide range of parameters.

The biological relevance of downregulating or abolishing the WR-Kir outward $I_{\text{res}}$ current as a parametric perturbation is directly suggested by (1) electrophysiological data in several disease models we listed (Tong, 2014), (Bataveljić, 2012), as well as (2) by the specific properties of heteromeric Kir4.1/Kir5.1 channel populations (Hibino et al., 2010), (Marmolejo-Murillo et al., 2021). The later accounts for a nonnegligible fraction of Kir4.1/Kir5.1 channels in astrocytes in different brain regions (Hibino, Fujita, Iwai, Yamada, & Kurachi, 2004), (Soe, Andreasen, & Klaerke, 2009), (Seifert et al., 2009). The extent of deregulation of the inward conductance of the Kir4.1 – a decreased slope in the I-V curve negative to $V_r$, is not detrimental for the observed qualitative changes since it doesn’t produce by itself the N-shaped I-V curve.

The dashed line in Fig. 7C represents a typical perturbation line of (13) combining (i) permanent deregulation of outward conductance of WR-Kir, with (ii) changing inward WR-Kir conductance in experimental conditions where an astrocyte is (iii) subjected to depolarizing current input $I_{\text{ext}}$.

Slow depolarizations induced by $K^+$ accumulation in extracellular space (ECS), and subsequently the positive shift of $E_K$ and $V_r$, suggest that either as a parameter, a transient perturbation, or a slow variable in an extended 3-dimensional model, [$K^+]$o could be inducing instability of $V_r$. Since an extension of the system (13) by incorporating [$K^+]$o dynamics in $R^3$ or higher (Barreto & Cressman, 2011) is beyond this study, we verified that fold and cusp are robust in ($I_{\text{ext}}$, [$K^+]$o) parameter domains as well, with $I_{\text{ext}}$ in the same range as above, and [$K^+]$o modestly raised compared to the nominal 2.5 mM, Fig. 8.

Large separation of time scales between $V_m$ responses and [$K^+]$o transients, from milliseconds to second and minutes, warrants analyzing the basic [$K^+]$o impacts on $V_r$ stability treating it as a parameter in the simple 2-dimensional system (13).

Quantitative studies summarizing the properties of macroscopic currents through the glial Kir4.1 (Seifert G., 2018) and K2P-TREK channels (Schewe et al., 2016), (Chen, 2014) suggest that apart from K2P activation there is no other channel kinetics critical to extend (13) with additional dynamical variable. Whole-cell Kir4.1 currents were showing rise times of less than 2 ms and negligible difference between the peak and steady current levels, in the mid-range voltages $V_m$ where the interaction of different currents is dynamically interesting. As a result, there is no candidate for a resonant variable in the 2-D model (13) (Izhikevich, 2007), (Izhikevich, 2004) which would induce a cyclic behavior in the minimal, 2-dimensional autonomous system (13).

3.2 Numerical simulations of the bistability and switching

For demonstrating the switching capability we perturbed the model (13) using time series from recordings of glial depolarization during electrographic seizures induced by transient elevation of [$K^+]$o (Traynelis & Dingledine, 1988). The glial $V_m$ was recorded using two-electrode in-situ protocol on glial membrane in rodent hippocampal slices.

Figure 9, (upper trace) shows a sample of glial depolarization $\Delta V_{\text{lfp}}$ referenced to an LFP of a near electrode, in response to neuronal seizure, Fig. 11A, in (Traynelis & Dingledine, 1988), digitized from a printed image using the Graph Grabber tool (Benbow, 2020). Such voltage generator signal
features: (i) a fast depolarizing, tonic transient of $\Delta V_m \approx 20\,mV$ within initial ten seconds, followed by (ii) pseudo-periodic clonic episodes lasting in total for nearly 60 seconds. To use the $\Delta V_{lfp}$ recording as a perturbing signal in our model (13) we need somewhat realistic time course of the corresponding $\Delta E_K(t)$ shift, for which purpose we used $\Delta V_{lfp-dc}$ averaged signal of 135 seizure events, the lower curve in Figure 9, see Fig. 10A, in (Traynelis & Dingledine, 1988), where as well the measurement electrode on glia has been referenced to the LFP immediately adjacent to the glial cell body, so that for $I_{ext}$ in (14) we get:

$$I_{ext}(t) = I_{gjc} + I_{lfp}(t) = I_{gjc} + g_{lfp}\Delta V_{lfp}(t) \quad (nA)$$
$$E_K(t) = E_K + \Delta E_K(t) \approx E_K + \Delta V_{lfp-dc}(t) \quad (mV)$$
$$\Delta V_{lfp-dc} = \langle \Delta V_{lfp} \rangle t - \langle \Delta V_{lfp} \rangle_o \quad (mV).$$

Perturbation signals as in (15) mimic a transient depolarization event of a hippocampal glial cell in situ, depolarized by a positive, outward current resulting from sustained seizure-like discharges invading the surrounding neurons in a larger region, slowly and transiently elevating, and restoring the nominal $[K^+]_o$ during 72.6 seconds. Therefore, we do not simulate bath application of a constant elevated $[K^+]_o$ from (Traynelis & Dingledine, 1988), where apart from some brief, transient local variations of $[K^+]_o$ it has been kept constant, but try to simulate more realistic transient depolarization which in reality comes with transiently altered local $\Delta E_K(t)$. We find $\Delta V_{lfp-dc}$ to be a good example of $\Delta E_K(t)$ assuming it represents an average of LFP events (a) already subjected to low-pass filtering of the ECS (Bedard, Kroger, & Destexhe, 2006), and (b) of comparable shape in the initial steady depolarization phase, time-aligned at the onset. Its maximal amplitude of 12.8$mV$ is close to the Nernstian $\Delta E_K = 13.7\,mV$, corresponding to a shift of $[K^+]_o$ from the nominal 5 mM in our data (the nominal I-V relations in the model), to 8.5 mM bath concentration robustly producing the seizures (Traynelis & Dingledine, 1988). The slower rise time in the initial 10 seconds, as well as the steady decay of the averaged $\Delta V_{lfp-dc}(t)$ reflect the action of the Na-K pump and other restorative mechanisms of ion homeostasis. Such $\Delta E_K(t)$ is therefore a safe choice for low to moderate $[K^+]_o$ increases on the timescale of seconds, even a more conservative one, producing slightly less depolarized $E_K$ shifts if we start from a baseline of 2.5 mM of external $K^+$. Unspecific conductance $g_{lfp}$ was introduced to convert the transient voltage perturbation $\Delta V_{lfp}(t)$ into current perturbation signal in (15). Indicative value range was set between 0.01 ± 0.0155 $\mu S$ to keep the total $I_{ext}(t)$ in simulations within the range of the fold bifurcation diagram, Fig. 7A, matching the dynamic range of the clonic discharges in $\Delta V_{lfp}$ of $5 \div 10\,mV$, from $t = 10\,s$ onwards, Fig. 8.

**Figure 9 – Transient glial depolarization signals** – Depolarization transient $\Delta V_{lfp}$ from resting (upper trace) measured on glial soma in situ, in response to an electrophoretic seizure initiated by increased $[K^+]_o$ to 8.5mM, in a rat hippocampal slice. A two-electrode protocol was used with a reference electrode in the bath. $\Delta V_{lfp-dc}$ (lower trace) is an average of 135 depolarizing episodes, recorded from 5 cells, used to approximate the shift of the Nernstian potential $\Delta E_K(t)$ due to $K^+$ accumulation.

A simulated example of switching behavior in response to the above perturbing transients is illustrated in Figure 10. Dotted lines are $V_m$ responses from ten solutions of (13) perturbed with $I_{ext}(t)$ with $0.01 \leq g_{lfp} \leq 0.0155$, $I_{gjc} = 0.2$, at $[K^+]_o = 2.5\,mM$ and $g_{s-inw} = 0.00917\, \mu S$, with outward WR-Kir current reduced to 10% of its amplitude. Qualitatively the glial $V_m$ is quenching the clonic discharges to a few millivolt variations, but around two different voltage baselines corresponding to the down-state or the up-state, depending on $g_{lfp}$ value. For $0.0137 \leq g_{lfp} \leq 0.014\, \mu S$, after the tonic transient, the responses do not follow the shape of the perturbation but
mark some form of boundary or a separatrix response or no-man-land of \( V_m \). Beneath and above that \( g_{\text{TP}} \) range the glial membrane responded with an expected waveform, either close to the down-state \( V_d \), or with a depolarized transient around the up-state \( V_{uT} \). The bold black lines show an average of ten runs with randomized \((g_{\text{TP}}, g_{\text{leak}})\) values within 10% variation, for each of the three qualitatively different responses. No particular sensitivity to either of the parameters was observed indicating a hidden peculiar singular behavior, in dynamical terms.

**Figure 10 – Glial switching between down-state and up-state was robustly observed for parameters in the bistability range of the resting state(s) in simulations of the model (13), perturbed with a seizure-like transient, Fig. 9. Dotted lines show ten simulations for \( 0.01 < g_{\text{TP}} < 0.0155 \mu \text{S} \). To verify the parameter ranges are robust to fluctuations, we randomized \((g_{\text{TP}}, g_{\text{leak}})\) values in ten runs of the three qualitatively different responses within 10% of their indicative ranges and averaged them, bold black lines. The up-state and down-state are separated by a separatrix response that does not follow the perturbing waveform. Varying \( I_{\text{GJC}}, g_{\text{leak}} \) and nominal \( [K^+]_o \) change the threshold and waveform but the two-plateau response typically remains for \( I_{\text{GJC}} \) in bistability range.**

The asymmetry of the division of \( g_{\text{TP}} \) range reflects the position of the saddle point \( V_s \) between the two stable nodes \((V_d \) and \( V_{uT})\), Fig.6B, or in other words, the position of the stable manifold of the saddle \( V_s \), which is the true separatrix in the phase plane.

4. **Discussion / I ~ importance, S – significance, N – novelty, U - utility /**

The observed dynamical instability could point to different areas of impact depending on the physical model system and the specific \( V_m \) dependence of the modeled phenomenon. A general conclusion in hypothesis-free biophysical terms would be that with its very basic ion channel composition the glial membrane responds differently and distinctly to external depolarizations when Kir or combinations of Kir and K2P conductances are deregulated, due to the interplay of Ohmic and complex non-Ohmic conductances, nonlinear in \( V_m \). Below, we discuss the possible implications of the presented biophysics from several different neurobiological perspectives.

(I1) There have been numerous association studies where changes in expression of Kir4.1 currents and the biophysical properties of measured macroscopic currents have been related, put in context, to the altered function of glia. In translating such changes into specific roles of the cell, it has remained elusive which mechanisms could transduce the molecular changes into a whole-cell response. We modeled \( V_m \) dynamic of an isolated astrocyte by a minimal ODE model incorporating the major \( K^+ \) conductances, WR-Kir and K2P, and a leak current to explore the direct effect of nonlinear changes in the total astrocytic ion conductance to \( V_m \). In other words, it models the simplest form of modulation of \( V_m \) by alterations of ion conductances with no other signal transductions being modeled, like membrane mechano-sensitivity, pH sensitivity, actions of physiological agonists, etc. The main question in the stability analysis of (13), is which nature of (de)regulation of the whole-cell conductance impacts the stability of the nominal resting state \( V_r \), which in turn controls all electricgenic transport mechanisms.

(I2) Decades of modeling using Hodgkin-Huxley conductance-based models suggest that even very small currents should be carefully considered since the nonlinear interplay of their conductances could change the stability of the resting state and in turn the cell response (Izhikevich, 2007). That has motivated the incorporation of the K2P current through its activation dynamics, \( n(t) \), as a second dynamical variable. The necessary model perturbations came from decreased Kir conductance in the outward \( V_m \) range, with K2P conductance remaining unchanged or eventually increased, resulting in a non-monotonic steady-state I-V curve. (N1) In that regard we present the
first ODE model of glial \( V_m \) (13) where two distinct and differentially regulated \( K^+ \) currents, in addition to the unspecific \( K^+ \) leak current, have been incorporated based on recordings from isolated cells.

(S1) Non-monotonous, N-shaped I-V relation produced by a prominent decrease of WR-Kir in the outward range, gives birth to a new, much more depolarized stable steady state \( V_{dr} \) for a physiological range of average external depolarizing current, \( I_{ext} \). When present, such a mathematical feature of a model of a non-excitable cell is generic and observed robustly in a wider parameter range, Fig. 7. We argue here that the emergence of a distinct and stable \( V_{dr} \) introduces a specific and distinctive transition - translating the loss of pseudo-Ohmic, linear behavior of the membrane into a bistable switch when exposed to depolarizing transients, Fig. 9. It would be unrealistic and insignificant to demonstrate that switching between \( V_r \) and \( V_{dr} \) is plausible in real glial cells by simulating the model using strong constant current perturbations \( I_{ext} \), since glial cells in real circuits do not receive prolonged depolarizations by constant external currents. The initial, steady depolarizing phase of the realistic \( V_m \), transient we used in (15), the first ten seconds before the clonic phase (Traynelis & Dingledine, 1988), is critical for the switching between the up-state and down-state to be observed and we believe has a typical waveform for a wider range of strong transient depolarizations resulting from local neuronal synchrony.

(S2), (N2) Translating \( V_m \) bistability and altered WR-Kir current into effects significant for the whole cell function, the following impacts are directly implicated by the model:

- **Depolarized resting state** \( V_{dr} \) as a catastrophic event\(^6\) – Even though it is one of the few primitive instability scenarios, the birth of a stable, very depolarized resting state is potentially catastrophic since switching to \( V_{dr} > -10 \text{ mV} \) is taking the membrane and the cell, to a state with much higher potential energy than the nominal hyperpolarized resting state, not corresponding to the actual \([K^+]_o\). Prolonged depolarization of the glial membrane around \( V_{dr} \) would impact all glial functions, from metabolic support to membrane transport, and practically all neuromodulatory roles assigned to glia.

- **(I3) Voltage switching between** \( V_r \) and **\( V_{dr} \) disrupts the kinetics of active ion and other transport mechanisms nominally running near** \( V_r \) - Since all major electrogenic pumps and transporters show some extent of voltage dependence (De Weer, Gadsby, & Rakowski, 1988), (Läuger, 1984), (Poulsen, Morth, Egebjerg, & Nissen, 2010), the depolarized \( V_{dr} \), or the up-state should violate the assumptions made for the steady-state transport rates near \( V_r \). In the case of ATP-driven pumps, in the Na-K pump, for example, all simplified descriptions used in computational models, in a form of a simple current generator \( I_{pump} = \rho f ([K^+]_o, [Na^+]_i) \), focusing on the “acceleration” term \( f \) which models the sensitivity to ion concentration changes, are warranted by the existence of a constant steady-state rate \( \rho \), assumed to be voltage-independent. When \( V_m \) switches to \( V_{dr} > -10 \text{ mV} \), dramatically shifted \( \Delta V_m \) to large positive values impacts some of the binding and translocation steps in the kinetic model of the pump and questions the biophysical plausibility of keeping \( \rho \) constant. In particular, in cases where the perturbing voltage transient like the electrographic seizure we used, produces a jump near \( V_{dr} \) not resulting from moderately elevated \([K^+]_o\), i.e. \( E_K \) not much above \( V_r \), a large and lasting positive transient is produced. If further studies prove the bistability in \( V_m \) is electrophysiologically plausible, moving to a more detailed macroscopic model of the pump might be required with \( \rho \) explicitly depending on the full form of the electrochemical potential. For the pump rate \( \rho \) in units of molar flux, we can write (Läuger, 1984):

\[
\rho \sim \Delta \mu + \Delta \mu_H \text{ (mM/s), where } \Delta \mu_H = \Delta \mu + zFV_m \text{ (RT units),}
\]

\[
RT \ln(c_{ext}/c_{in}) + zFV_m < -\Delta \mu, \text{ for active transport, (16)}
\]

\(^6\) We use the term in the context of the catastrophe theory of dynamical systems (Gilmore, 1993), (Zeeman, 1977) and catastrophic bifurcations (Abraham & Shaw, 1982), initially formulated over the impact of the same instability we observed - the fold bifurcation, on the global behavior of both, natural and man-made systems.
where \( \Delta G \) represents the purely chemical potential energy from ATP hydrolysis, and within the electrochemical part \( \Delta \mu \), we recognize the Nernstian, or osmolar contribution \( \Delta \mu = RT \ln \left( \frac{c_{\text{ext}}}{c_{\text{in}}} \right) \) and the electrical part \( zFV_m \) that impacts \( \rho \) under prolonged depolarization at \( V_{dr} \). An implicit assumption not typically discussed is that only near the nominal equilibrium \( V_e \), where the electrochemical gradient \( \Delta V_m \) is small and the osmolar contribution large and steady, the \( FV_m \) term could be eventually neglected. The inequality in (16) is the typical condition for a sustained primary active transport, with sign convention in \(-\Delta G\) representing the amount of free energy available from hydrolysis of a mole ATP, rather than the barrier height in the enzymatic reaction (Adam, 2009), (Läuger, 1984). Some variants of the macroscopic model of the pump have kept the voltage dependence (Forrest, 2014) and have produced effects in whole-cell dynamics attributable to the Na-K pump. In such models, the eventual effects of voltage bistability could be directly tested. Stoichiometry coefficients have been omitted in (16) for simplicity.

The electrogenic sodium bicarbonate \( Na^+/HCO_3^- \) cotransporter (NBCe1) specifically implicated in glial metabolic and other functions, is another important candidate to be analyzed in the context of bistability since the reversal of the polarity of \( \Delta V_m \) would cause switching of the transport direction and in turn the whole-cell mode between alkalinization and acidification of the cell itself, and consequently the immediate extracellular environment (Ransom, 2000).

Of the other transporters potentially “vulnerable” to bistability let us mention the astrocytic GABA transporters (GAT1-GAT3) expressed in local cortical circuits (Fattorini, Melone, & Conti, 2020), (Zafar & Jabeen, 2018), modeled macroscopically as explicitly \( V_m \) dependent (Hoshino, Kameno, Kubo, & Watanabe, 2020).

- (4), (S3) Glial encoding of \([K^+]_v\) variations as a signal gets a distinct state – In the present understanding of the encoding capability of glial membrane, the glial \( V_m \) with certain low-pass filtering capability (with \( \tau_m = C_{\text{eff}} / g_{\text{stope}} \approx 10\ ms \) for 20pF patch capacitance) close to linearly encodes \([K^+]_v\) variations as a signal through \( (V_m - E_K) \). We can also say \( E_K \) itself smooth out the real-time \( K^+ \) variations from neuronal discharging partly due to the low-pass filtering of the ECS to volume transmission signals (Bedard et al., 2006) and partly due to the \( \log \left( \frac{c_{\text{out}}}{c_{\text{in}}} \right) \) nature of Nernstian dependence. The emergence of \( V_{dr} \) introduces therefore a distinct, depolarized state potentially detectable on various timescales by a suitable measurement setup. In other words, \( V_e \) and \( V_{dr} \) encode the (a) almost linear potassium electrode, and (b) the deformed, N-shaped I-V relationship of the deregulated membrane, respectively, Fig. 6A.

- (5), (S4) Switching between the two resting states may introduce erratic transients in trans-junctional voltage over the GJCs – It has been shown that the conductance and rectification of GJCs are dependent on the trans-junctional voltage \( V_j \) between the connected cells across a wide range of the Connexin isoforms (Bargiello et al., 2018), (Oh, 2015), (Maciunas, Snipas, Paulauskas, & Bukauskas, 2016), and specifically for the Connexin43 hemichannels, one of the two most abundant subtype assembling the astrocytic GJCs, with Connexin30 (Bukauskas, Bukauskiene, & Verselis, 2002). In addition to the nonlinear steady-state I-V relationship of the GJ connections and \( g_{gjc}(V_j) \) dependence of the conductance, the complex two-stage gating in most of the hemichannels may result in erratic behavior of the GJC if the connected cells are prone to switching between \( V_e \) and \( V_{dr} \). Atomic level model of GJC electrostatics (Escalona et al., 2016) suggests complex Coulombic profiles potentially vulnerable to erratic \( V_j \) and \( V_m \) switching on multiple positions. The resulting \( V_j \) and \( V_m \) switching may keep both, the hemichannel and the GJC dwelling in an unstable state between the residual and fully conductive conformations, or depending on the frequency of bistability switching between the instantaneous and steady \( g_{gjc}(V_j) \) which could be markedly different (Oh, 2015). Even though massive glial disconnection is a proven hallmark of chronic epilepsies (Bedner et al., 2015), (Binder & Steinhauser, 2021) eventual
The causative role of GJC failure is yet to be shown pointing eventually to the biophysical mechanism.

In the case of open, background Kir channels we departed from the typical modeling approach, a single current – single biophysical description. The reversal of polarity of the driving force changes the nature of the physiological block resulting in qualitatively different pseudo-gating in the WR-Kir pore for inward and outward flux (Appendix A). This assumption warrants to use two different biophysical descriptions for additive inward and outward fluxes. Figure 3 suggests that this conceptual approach results in an overlapping $V_m$ regions where both mechanisms (blue and red curve in Fig.3) produce a small outward current. Those outward permeation events happen for very small $\Delta V_m$ where the electrical enthalpic contributions are comparable to the fluctuations – resulting in outward pseudo-gating in both, the central cavity, as well as within the SF, described respectively by the Hagiwara model, and by the extended, non-classical GHK equation (5). Such different gating scenarios require several effective charge valences $z_{\text{inv}}, z_B, z$ to appear within descriptions of WR-Kir current.

(N3) Departing from the constant field assumption in the Nernst-Planck formalism of an open pore, results in a modified Goldman-Hodgkin-Katz current equation which allows incorporating simple forms of nonlinear energy profiles $U(x)$ within the pore, as long as the integral in the current equation is solvable (Jackson, 2006). We applied it for the first time to describe the outward component of WR-Kir current (8), and by extending it with quadratic dependence of the barrier peak (7), the observed current saturation with voltage was captured by the model, Fig. 4. Accumulating evidence of complex gating in open pores, that does not express a voltage-sensitive gate, may qualify this approach in a wide range of leaky channels.

In summary, the proposed minimal 2-dimensional model of the interplay between WR-Kir and K2P currents in astrocytic membrane shows that deregulations turning WR-Kir current into inwardly rectified, robustly produce a second very depolarized steady state of $V_m$ and a whole-cell behavior considered markedly non-physiological within glial biology. To prove or refute such bistability as neurobiologically plausible, a measurement protocol well suited for transient $V_m$ changes would be needed, probably employing voltage imaging, a dynamic (current) clamp, and cell assays displaying deregulated Kir4.1 or sufficient densities of Kir4.1/Kir5.1 channels of glial origin.

Preliminary extension of (13) we attempted with $[K^+]_o$ dynamics (Barreto & Cressman, 2011), (Cressman, 2009), (Krishnan, Filatov, Shilnikov, & Bazhenov, 2015) in a very simplified, spatially constraint ECS compartment demonstrated enriched dynamical repertoire of the model and will be reported in another study. Within $[K^+]_o$ ranges indicating the richer bifurcation repertoire in (Barreto & Cressman, 2011) and (Erhardt, Mardal, & Schreiner, 2020), we observed some entrainment of the glial $V_m$ in (13) by the $[K^+]_o$ dynamics driven by the neuronal bursting (Barreto & Cressman, 2011), but those require more precise dynamical characterization. Other important extensions of the model should include (a) incorporating the chloride conductances explicitly and (b) adding mechanosensitivity to K2P conductances to simulate the osmolar stress of the membrane.

Very limited availability of transient recordings from glia leaves us without traces of real-time perturbations of glial $V_m$ on different time scales which is a limiting factor to considering a non-autonomous, driven form of the model (13), since in immediate proximity of spiking neurons the glial cells are never in a true voltage steady state. The heavily interconnected glial cells in the glial cell continuum require testing any indications of $V_m$ multistability using boundary value problem models in the light of experimentally observed $V_m$ isopotentiality (Ma, 2016).

The presumed ion-homeostatic role of Kir4.1 in glia was not discussed in the context of bistability because the differential contributions of the different ion transport mechanisms are still elusive (MacAulay, 2020), and reasonably minimal mathematical model addressing the spatial complexity of volume transmission in neuroglial circuits requires a dedicated experimental study.

(U1) The proposed model (13) is of general biophysical utility, directly applicable to the interplay between Kir and K2P currents in membranes of other systems where K2P channel variants display nonnegligible activation kinetics, like in the heart and kidneys, see (Sepúlveda, 2015) for more
cases. Bistability showing comparable voltage separation of $V_r$ and $V_{dr}$ like presented has been already observed in cardiomyocytes in hypokalemic conditions (Zuo, 2017), where strongly-rectifying Kir2.1 channels interwork with the linear K2P-TWIK channels.

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The XPPAUT .ode files for the bifurcation analysis and simulations will be available in ModelDB at https://senselab.med.yale.edu/ModelDB, under the model name “Glial voltage dynamics driven by Kir & K2P currents”.

**Appendix-1 Short-pore model of WR-Kir channel and physiological $Mg^{2+}$ block**

We base our biophysical reasoning on (i) conduction phenomena through a short pore (Fig. A1), common to all $K^+$ channels - confined to the part of the transmembrane structure consisting of a selectivity filter (SF) and internal water-filled central cavity (Doyle et al., 1998), (Allen, Bliznyuk, Rendell, Kuyucak, & Chung, 2000) and on (ii) physiological blocking of the pore by cytoplasmic cations as a permeability control mechanism. Coulombic interactions within the short pore are therefore used to explain the permeation mechanisms as an interaction between the blocking $Mg^{2+}$ and permeant $K^+$ ions, as a basis for a weak rectification. Unlike in strong inward rectification, where both, the internal $Mg^{2+}$ and polyamines fully block outward flux (Lu, 2004), (Matsuda, 1991), in the outward permeation scenario of a weakly rectifying pore (Fig. A1C) the partial and flickering block allows the entrance of permeant ions into the SF at high rates. This pore model is crucial for explaining the anomalous, inward rectification of $K^+$ conductivity, which can't be described by Nernst-Planck (NP) formalism based on electrochemical gradients over open, leaky channels. The short pore model applied to outward conduction in WR-Kir channels, allows extending the NP model with a non-constant field and in turn a voltage-dependent permeability, equation (9), under the assumptions of a changing Coulombic profile with voltage. These extensions formulate the main assumptions of the $I_{res}$ model (Jackson, 2006).

Recent evidence from single-channel recordings and molecular dynamics simulations of the potassium KcsA pore (Heer, 2017) suggests that the voltage and ligands induce structural fluctuation changes within the electrostatics, Coulombic profile of SF which produces a universal pseudo-gating of an open pore at SF, without introducing a conformational change and gating.
The short-pore model assumes the concentration of \( M^2+ \) and \( K^+ \) within the central cavity and at the external pore vestibule, correspond to the physiological intracellular and extracellular bath concentrations respectively. Local, relative, nonequilibrium concentration changes due to effects like the attraction of cations by rings of negatively charged residues, in vestibules on either side of the SF entrance, are neglected.

We assume that the transmembrane voltage \( V_m \) drops only over short pore, mainly along the cavity and the SF, from S1 to S5 (which corresponds to the fractional electrical distance = 1), influencing with a smaller fraction the S5 position, marked \( \Delta V_m \) on Fig.3. S0 indicates a position just outside the SF, in the outer vestibule (Fig. A1B and A1C). This is a similar approach to the one used in permeation studies of weak rectification in renal Kir1.1 / ROMK1 (Yang, Edvinsson, Sackin, & Palmer, 2012b; Yang, Edvinsson, Sackin, & Palmer, 2012a). This pore model suggests that the blocking cation moves between two modulatory sites (B. Hille, Schwartz, W, 1978) depending on \( \Delta V_m \) polarity, thereby defining the main rectification barrier(s).

**Inward pseudo gating** - For negative and very small positive net driving forces \( \Delta V_m \), (Fig. A1B), the resulting electrostatics from pore helix macro dipoles keeps blocking \( M^2+ \) ions within the cavity (designated by position S6), so that the inward rectification results from a competition of permeant \( K^+ \) and \( M^2+ \) ions for the central, axial positions within the cavity. Even though \( V_m \) influences S6 position with a much smaller fraction \( \Delta V_m \) (Fig. 3) the dependence of inward flux on \( V_m \) for \( \Delta V_m < 0 \) is still exerted on the \( K^+ \) ion in S6 by the multi-ion file within SF (binding positions S1 to S4), which contributes to the resulting Coulombic forces and ion-crowding within the central cavity (represented by S5 and S6 positions). By resulting force, in this simplified view, we mean Coulombic repulsion between the permeant ions acting in parallel to the stabilizing attractive force on them coming from the negative end of pore helix macro dipoles (Fig. A1A), tilted so to make the center of the cavity electrostatically favorable point for a cation (B. Hille, 2001). Hagiwara’s measurements of macroscopic Kir currents demonstrated the existence of equilibrium pore-open probabilities of the inwardly permeant pore in Kir channels, suggesting the Boltzmann term in the Hagiwara model (3) for a description of macroscopic conductance, similar to open and closed probability in gated ion channels, see (Lu, 2004) for Kir-specific review.

**Outward pseudo gating** - In the reverse case, for a more positive driving force \( V_m > -50 mV \) (Fig. A1C), an outwardly directed net driving force acts on permeant and blocking cations, pushing partly or fully dehydrated \( M^2+ \) ion towards the entry of the SF, which results in a partial flickering plug of the pore at the S5 site. Blocking ion at S5 creates the entry barrier to the permeant ion, wide in units of fractional electrical distance (Fig. A1C), extending through S4 and S3 positions.

The different nature of the resulting electrostatics in these two cases implicates different permeation mechanisms and warrants the use of a different biophysical description of unidirectional fluxes. We, therefore, model the unidirectional fluxes as additive over the whole \( V_m \) range of the measured Kir4.1 current. In other words, the proposed modeling approach suggests that in its structure-function a weak rectifier results as a superposition of inward and outward rectifiers. Single mutation studies (Wible et al., 1994), (Lu Z., 1994) suggested the structural determinants are very similar and a single point mutation flips the pore from a strong Kir2.1 to a weak Kir1.1 rectifier.
### Appendix-2

#### Table 2 - Experimental studies reporting altered properties of Kir4.1 current

<table>
<thead>
<tr>
<th>Altered current</th>
<th>Exp. Model, Reference</th>
<th>WR-Kir inward</th>
<th>WR-Kir outward</th>
<th>Cell type</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kir4.1 current</td>
<td>Astrocytes in ALS (Bataveljić, 2012)</td>
<td>↓↓</td>
<td>↓↓</td>
<td>Astrocytes in culture</td>
<td>Non-Kir outward current increased markedly in the ALS model</td>
</tr>
<tr>
<td>Kir4.1 outward current</td>
<td>Astrocytes in Huntington's Disease (Tong, 2014)</td>
<td>↓</td>
<td>↓↓↓</td>
<td>Astrocytes in slices</td>
<td></td>
</tr>
<tr>
<td>Currents through the heteromeric Kir4.1/Kr5.1 channels</td>
<td>(Marmolejo-Murillo et al., 2021)</td>
<td>Not altered</td>
<td>Abolished</td>
<td>HEK-293 cells</td>
<td>Kir channels introduced by DNA transfection</td>
</tr>
<tr>
<td>Kir4.1 currents</td>
<td>Astrocytes in Depression (Cui, 2018)</td>
<td>↑↑</td>
<td>↑↑↑</td>
<td>Astrocytes in slices</td>
<td>Not considered in the parametric analysis since the I-V curve remains monotonic.</td>
</tr>
</tbody>
</table>
References


