SUPPLEMENTARY MATERIAL

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Methods for quantification of pore – voltage-sensor interaction in Ca$_V$1.2

Beyl S, Kügler P, Hohaus A, Depil K, Hering S and Timin E

Corresponding author: Steffen Hering

address: Austria, Vienna 1090, Althanstrasse 14, Department of Pharmacology and Toxicology
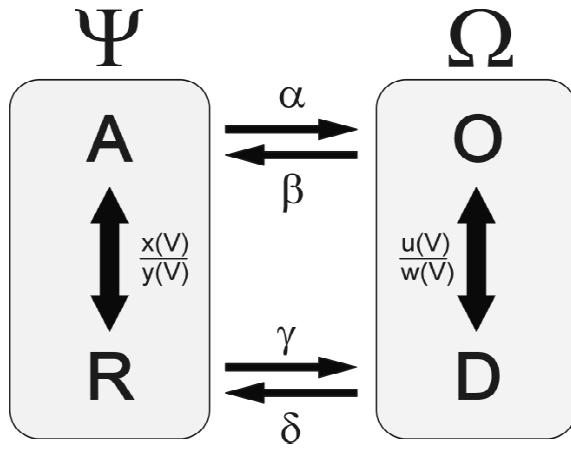
telephone: +43 1 4277 55310

fax: +43 1 4277 9553

e-mail: steffen.hering@univie.ac.at

Reduction of the 4-state circular model

In accordance with a concept of "the rate limiting stage" the fastest transitions might be analysed as being in quasy equlibrium condition. In our model we assume that these fast transitions are R↔A and O↔D:

$$R \cdot x(V) \cong A \cdot y(V)$$
$$D \cdot u(V) \cong O \cdot w(V)$$

/1/

We denote
$$\Psi = A + R$$
$$\Omega = O + D$$

/2/

From equations /1/ and /2/:

$$R = \Psi \frac{1}{1+\dfrac{x}{y}}, \ \ A = \Psi \frac{1}{1+\dfrac{y}{x}}, \ \ O = \Omega \frac{1}{1+\dfrac{w}{u}}, \ \ D = \Omega \frac{1}{1+\dfrac{u}{w}}$$

Transition from closed ($\Psi = A + R$) to open ($\Omega = O + D$) states:

$$\frac{d\Omega}{dt} = \alpha \cdot A - \beta \cdot O + \gamma \cdot R - \delta \cdot D =$$

$$= \alpha \cdot \Psi \frac{1}{1+\dfrac{y}{x}} - \beta \cdot \Omega \frac{1}{1+\dfrac{w}{u}} + \gamma \cdot \Psi \frac{1}{1+\dfrac{x}{y}} - \delta \cdot \Omega \frac{1}{1+\dfrac{u}{w}}$$

Condition $\Omega + \Psi = 1$ yields:

$$\frac{d\Omega}{dt} = \left[\frac{\alpha}{1+\dfrac{y}{x}} + \frac{\gamma}{1+\dfrac{x}{y}}\right] - \left(\frac{\alpha}{1+\dfrac{y}{x}} + \frac{\beta}{1+\dfrac{w}{u}} + \frac{\gamma}{1+\dfrac{x}{y}} + \frac{\delta}{1+\dfrac{u}{w}}\right)\cdot\Omega \qquad /3/$$

This equation predicts a mono-exponential time course with a time constant:

$$\tau_m = \left(\frac{\alpha}{1+\dfrac{y}{x}} + \frac{\beta}{1+\dfrac{w}{u}} + \frac{\gamma}{1+\dfrac{x}{y}} + \frac{\delta}{1+\dfrac{u}{w}}\right)^{-1} \qquad /4/$$

where $\dfrac{y}{x} = \dfrac{y_0}{x_0}\cdot\exp(-\dfrac{V}{k_S})$, $\quad \dfrac{w}{u} = \dfrac{y_0}{x_0}\cdot\dfrac{\beta}{\alpha}\cdot\dfrac{\gamma}{\delta}\exp(-\dfrac{V}{k_S})$ (see [4])

The steady-state activation curve is described as in Beyl et al. [4]:

$$m_\infty = \frac{1}{1+\exp\left(\dfrac{V_m - V}{k_S}\right)} \qquad /5/$$

with midpoint: $V_m = V_X - k_S \cdot \ln\dfrac{1+\dfrac{\alpha}{\beta}}{1+\dfrac{\gamma}{\delta}} \approx V_X - k_S \cdot \ln\left(1+\dfrac{\alpha}{\beta}\right) \qquad /6/$

and slope factor $k_S = \dfrac{k_x \cdot k_y}{k_x + k_y}$ and $V_X = k_S \cdot \ln\left(\dfrac{y_0}{x_0}\right)$. $\qquad /7/$

Fopmulae /4/ - /7/ were used for estimation of the model parameters.

Asymptotic estimation of the model parameters

Asymptotic estimations of voltage dependence of τ_m yields:

At strong depolarization ($V \to +\infty$):

x/y → +∞ and u/w → +∞

y/x → 0 and w/u → 0 that yields

rate(depolarization) = 1/τ_m(*depolarization*)→ α + β

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At strong hyperpolarization (V → -∞):

   y/x → +∞ and w/u → +∞

   x/y → 0 and u/w → 0 that yields

   $rate(hyperpolarization) = 1/\tau_m(hyperpolarization) \rightarrow \gamma + \delta$

Figure 1 illustrates that all items in the sum *rates* reach their asymptotic values at physiological

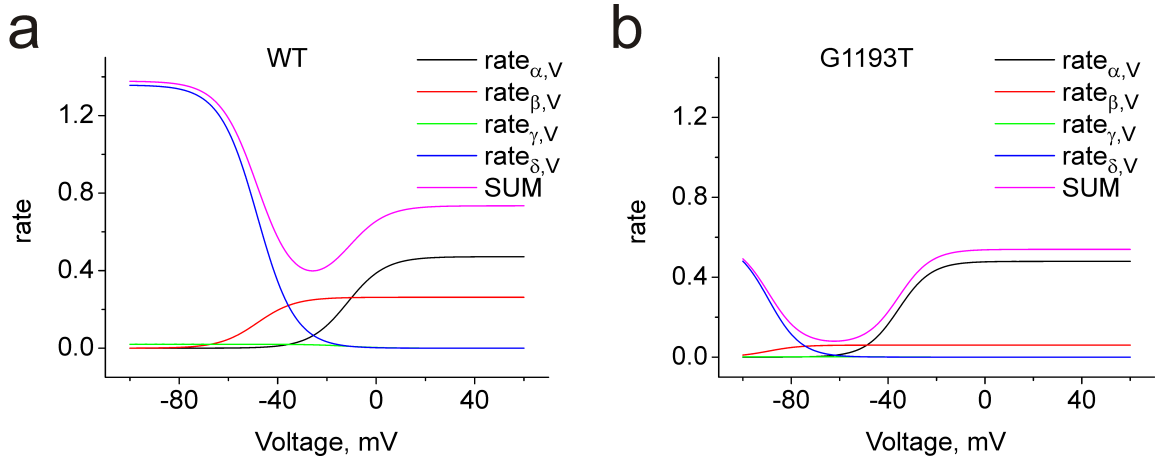membrane voltages (exemplified for wild type and mutant G1193T).

Figure 1. Impact of different transition rates on voltage dependent activation/deactivation exemplified for WT (A) and G1193T (B). The corresponding rates are given by

$$rate_{\alpha,V} = \frac{\alpha}{1+\frac{y(V)}{x(V)}} ; rate_{\beta,V} = \frac{\beta}{1+\frac{w(V)}{u(V)}} ; rate_{\gamma,V} = \frac{\gamma}{1+\frac{x(V)}{y(V)}} ; rate_{\delta,V} = \frac{\delta}{1+\frac{u(V)}{w(V)}}$$

At V = V_m the impacts of particular rates into the resulting rate are (formulae deduced under

assumption that γ <<δ):

$$rate_{\alpha,V} \approx \frac{\alpha}{2+\frac{\alpha}{\beta}} , \ rate_{\beta,V} \approx \beta , \ rate_{\gamma,V} \approx \frac{\gamma(1+\frac{\alpha}{\beta})}{2+\frac{\alpha}{\beta}} , \ rate_{\delta,V} \approx \gamma(1+\frac{\alpha}{\beta})$$

Major impact into resulting rate (sum of particular rates) is introduced by $rate_{\beta,V}$. Thus, time

constant at potentials near the middle of the activation curve (V_m) is approximately equal to 1/β.

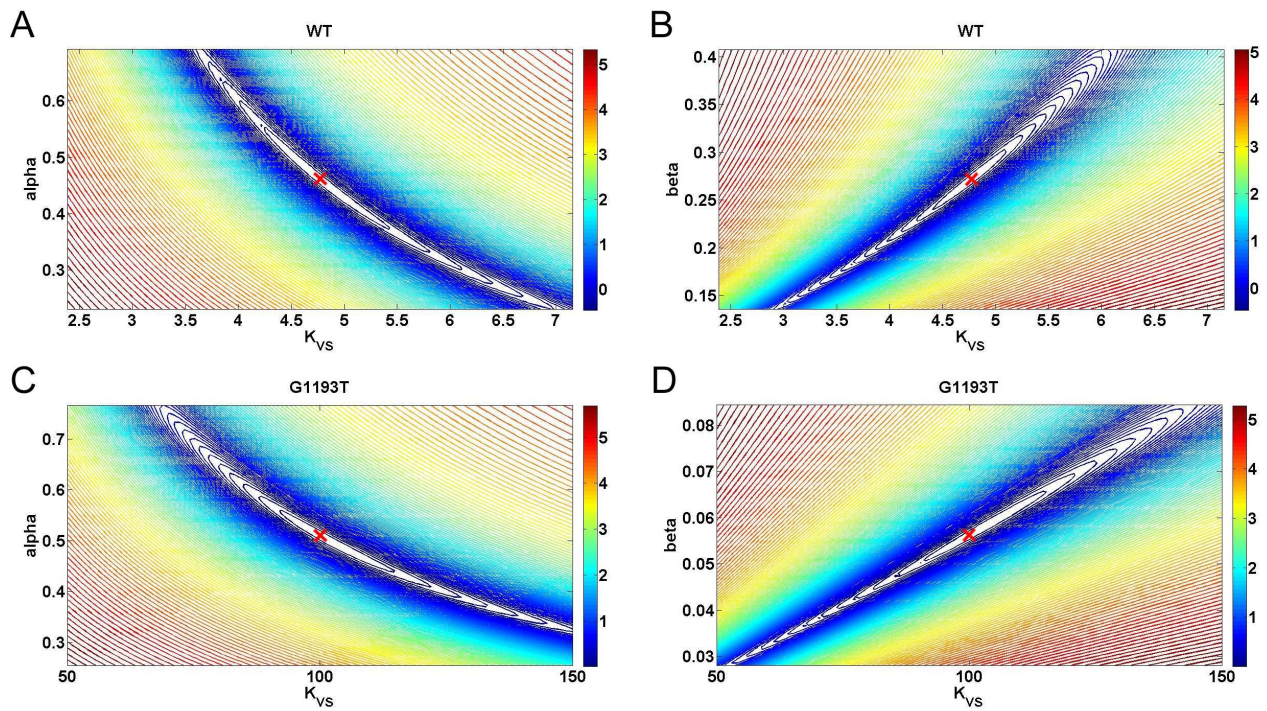*Illustration of the unique identification of the model parameters*

Figure 2. Graphical illustration of parameter identifiability

Plot shows the level sets of the logarithm of the objective function *J(q)* (see eg. /1/ in Results section) in the neighborhood of the identified parameter marked by the cross (center of the graph). Two out of the six model parameters are varied according to the axis labels, the remaining parameters are fixed by their values. The closed lines indicate pronounced curvatures in the parameter landscape and hence (at least local) uniqueness of identified parameters. Similar observations are made for other parameter variations.