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Supporting Material

A stochastic four-state model of contingent gating of gap junction channels containing two 'fast' gates sensitive to transjunctional voltage

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Supplement – 1

Shown in Figures S-1, S-2 and S-3 are the screen captures obtained by simulating voltage gating using three different V_j-protocols: 1) consecutive V_j steps rising in the amplitude, 2) slowly rising V_j ramps, and 3) series of V_j steps. Fig. S-4 shows g_j, I_j and V_j plots over time obtained by simulating g_j-V_j dependence shown in Fig. 7A. In all presented examples the junction comprised 1000 GJ channels.

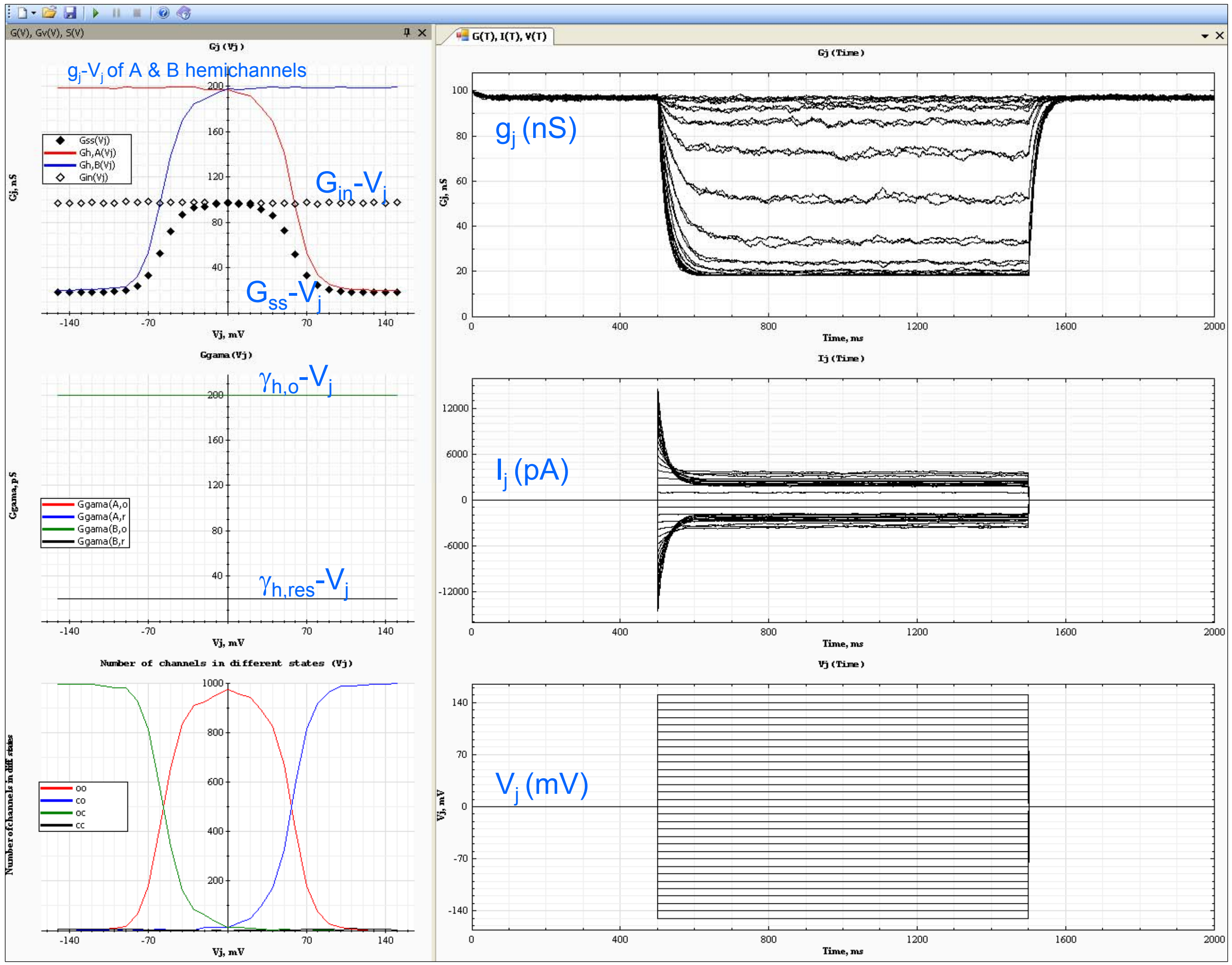

Fig. S-1. V_j-gating in GJs simulated by applying consecutive V_j steps increasing in amplitude, ΔV_j= 10 mV. Simulation was performed using following parameters: $\gamma_{h,o}$=200 pS, $\gamma_{h,res}$=20 pS, $V_{h,o}$=40 mV, A_h=0.1 mV^{-1}, $\varpi_o=\infty$ and $\varpi_{res}=\infty$. Calculation time, ~12 s.

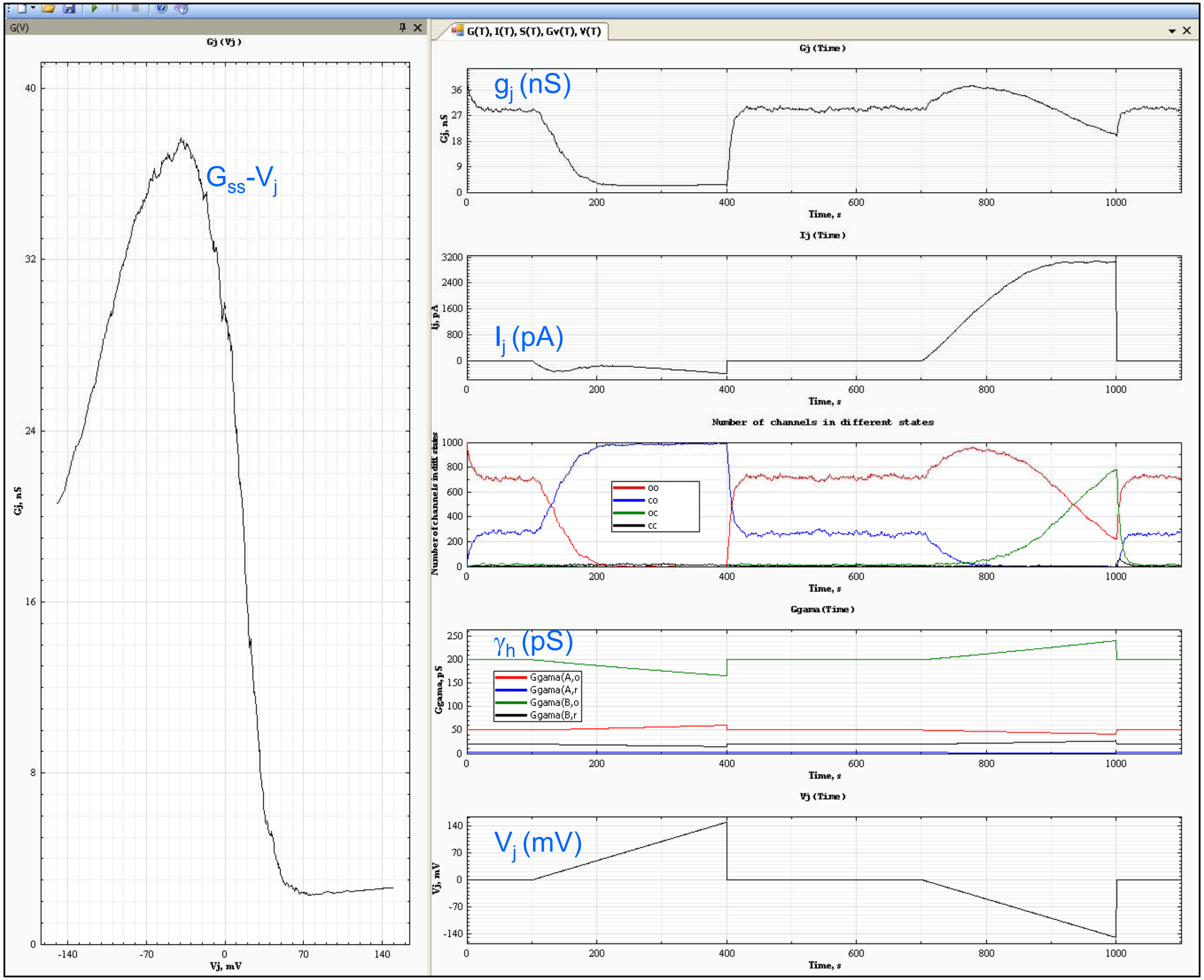

Fig. S-2. V_j-gating in heterotypic junction simulated by applying slowly rising V_j ramps. Parameters for hemichannel A: $\gamma_{hA,o}$ =50 pS, $\gamma_{hA,res}$=2 pS, $V_{hA,o}$=10 mV, A_{hA}=0.1 mV^{-1}, $\varpi_{hA,o}$=800 mV and $\varpi_{hA,res}$=500 mV. Parameters for hemichannel B: $\gamma_{hB,o}$ =200 pS, $\gamma_{hB,res}$=20 pS, $V_{hB,o}$=40 mV, A_{hB}=0.1 mV^{-1}, $\varpi_{hB,o}$=800 mV and $\varpi_{hB,res}$=500 mV 11. Calculation time, ~2 s.

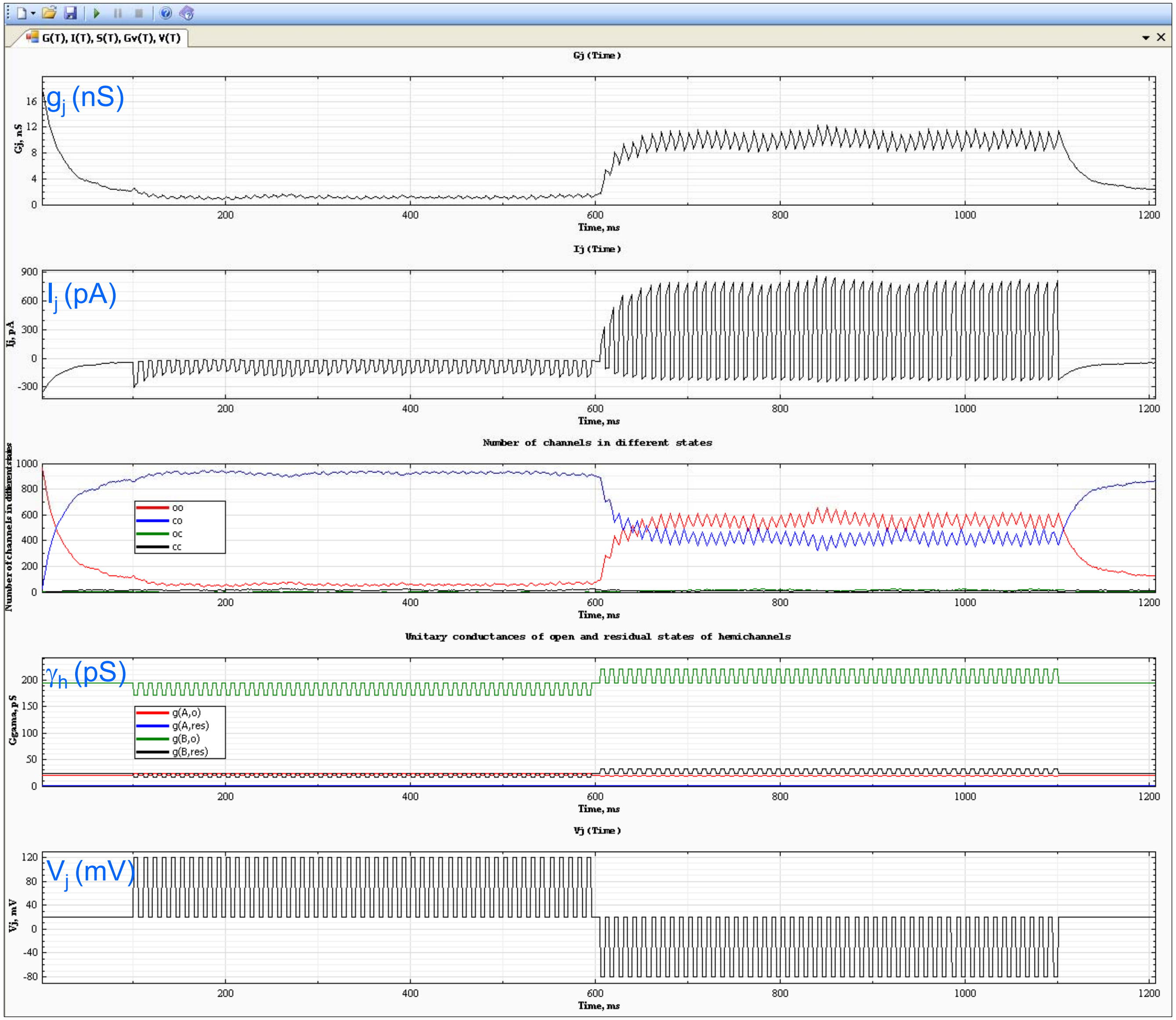

Fig. S-3. Simulation of signal transfer asymmetry in a heterotypic junction using series of negative and positive V_j steps. Duration and amplitude of individual steps were 5 ms and 100 mV, respectively. Parameters for hemichannel A: $\gamma_{hA,o}$ =20 pS, $\gamma_{hA,res}$=1 pS, $V_{hA,o}$=1 mV, A_{hA}=0.1 mV^{-1}, $\varpi_{hA,o}$=800 mV and $\varpi_{hA,res}$=300 mV. Parameters for hemichannel B: $\gamma_{hB,o}$ =200 pS, $\gamma_{hB,res}$=25 pS, $V_{hB,o}$=40 mV, A_{hB}=0.1 mV^{-1}, $\varpi_{hB,o}$=800 mV and $\varpi_{hB,res}$=300 mV. Calculation time, ~2 s.

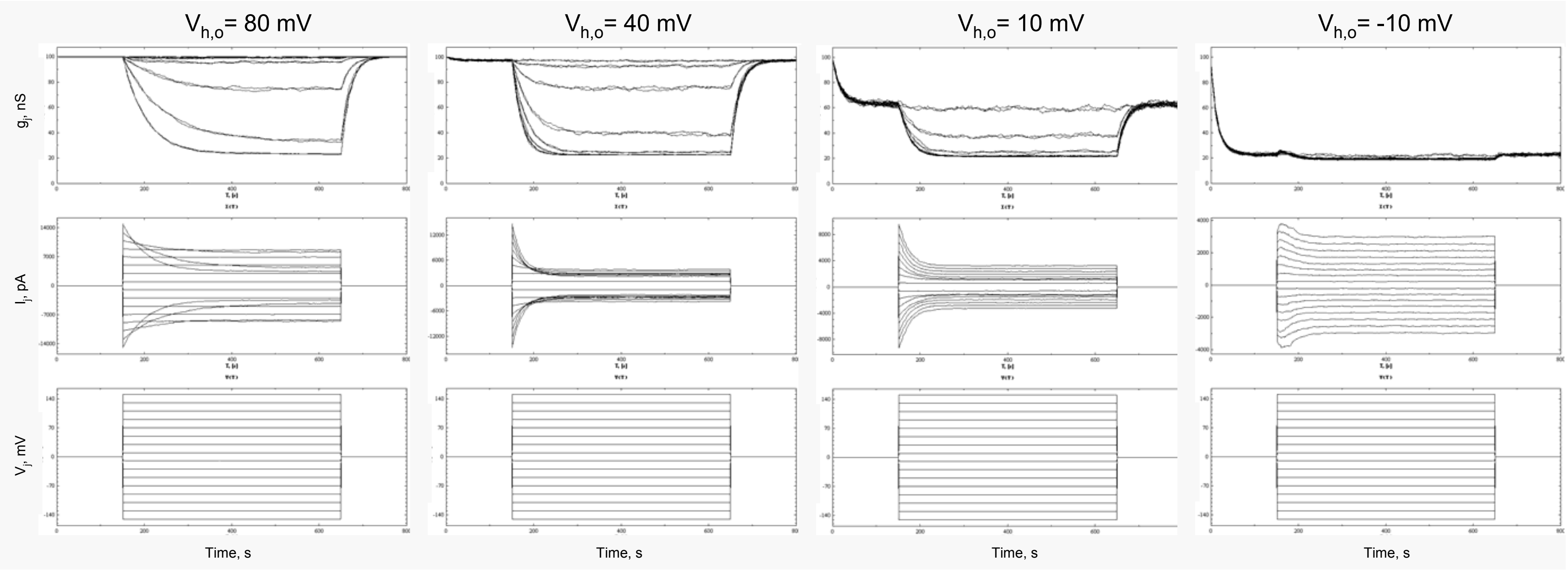

Fig. S-4. Shown are g_j, I_j and V_j plots over time obtained by simulating g_j-V_j dependence shown in Fig. 7A at four different $V_{h,o}$s: 80, 40, 10 and -10 mV. V_j-gating was examined using consecutive V_j steps rising in amplitude by 20 mV.

Supplement-2

Description of aggregates used to elaborate an algorithm for the analysis of voltage-gating properties of gap junction channels.

The model is realized using piece-linear aggregate (PLA) formalization method [1,2]. Formalized system is represented as a set of aggregates, $S = \{A_1, \ldots, A_n\}$. Each aggregate includes:

1. Input signals: *X*.
2. Output signals: *Y*.
3. External events: E'.
4. Interval events: E''.
5. Controlling sequences, which define time instances when internal events occur.
6. Parameter z(t) that describes the state of the aggregate, $z(t) \in Z$. State $z(t)$ consists from discrete $\nu(t)$ and continuous $z_\nu(t)$ components:
$z(t) = (\nu(t), z_\nu(t))$.
7. Initial state
$z(t_0) = (\nu(t_0), z_\nu(t_0))$.
8. Transition operators: $H(e_i)$, $e_i \in E' \cup E''$. These operators describe change of the state of aggregate after event e_i. $H(e_i): Z \times (E' \cup E'') \to Z$.

9. Output operators: $G(e_i), e_i \in E' \cup E''$. These operators describe output signals which occur after event e_i.

$$G(e_i): Z \times (E' \cup E'') \to Y.$$

Links between aggregates are described by the relationship:

$$R : \bigcup_{i=1}^{n} \{X_i\} \to \bigcup_{i=1}^{n} \{Y_i\}.$$

1. Conceptual model

Let us assume that a gap junction (GJ) plaque is composed from n GJ channels, C_i ($i \in \{1,2,\ldots n\}$) (Fig. 1). GJ channel consists of two hemichannels each containing the gate, a_j ($j = \overline{1,2}$) (Fig. 2). Every gate, $a_{i,j}$, has conductance $g_{i,j}$, which is derived from the transjunctional voltage (*U*) and the state of the gate ($s_{i,j}$). It is assumed that voltage across the hemichannel is equal to voltage across the gate.

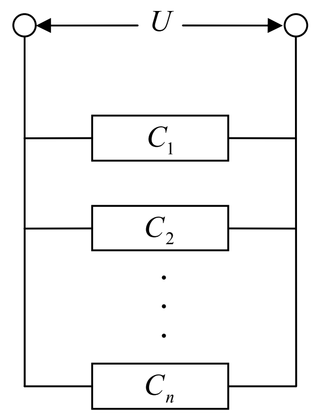

Fig. 1. Schematics of the system composed of *n* GJ channels arranged in parallel fashion.

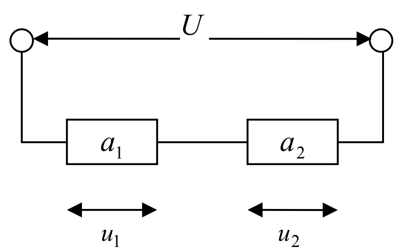

Fig. 2. Schematics of a GJ channel that consists of two hemichannels containing gates, a_1 and a_2, sensitive to voltage across each of them, u_1 and u_2 ($U = u_1 + u_2$).

An example of U changes over time is shown in Fig. 3.

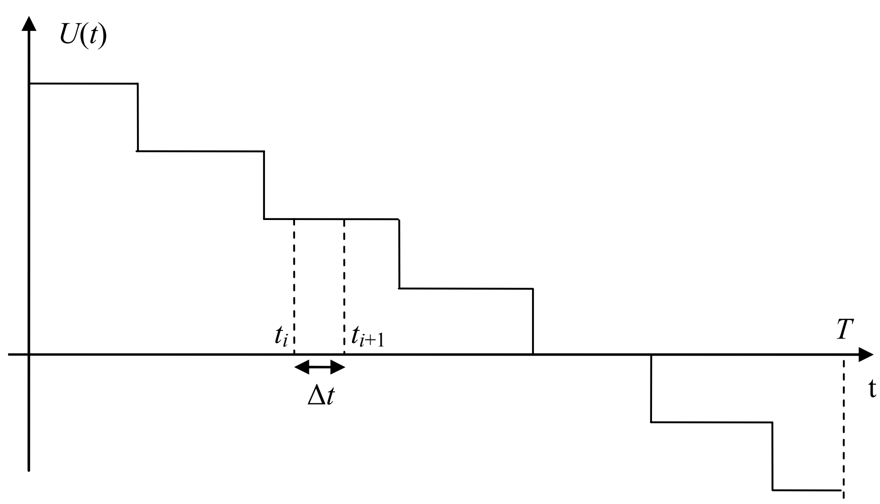

Fig. 3. An example of stepwise U changes over time.

Each gate can be in two states: o – open or c – closed (Fig. 4). Change in the gate state can occur at the discrete moments. Probabilities of transitions depend on $u_j(t)$, $j = \overline{1,2}$, $t \in [0, T]$.

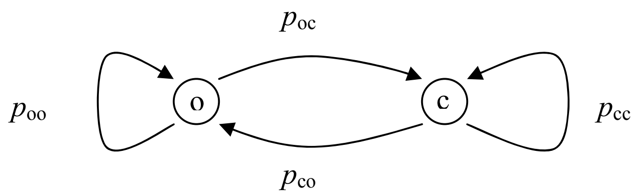

Fig. 4. Schematics of open and closed states of the gate. p_{oc} and p_{co} are probabilities of transitions between the states; and p_{oo} and p_{cc} are probabilities to remain in the same state.

Conductance of 'left' and 'right' hemichannels (g_l and g_r) depend on the state of their gates ($s_l(t)$ and $s_r(t)$; $s_l, s_r \in \{o, c\}$) and voltage across them ($u_l(t)$ and $u_r(t)$; $t \in [0, T]$) to account conductance rectification:

$$g_l = g(s_l(t), u_l(t)) \text{ and } g_r = g(s_r(t), u_r(t)) .$$

Then, macroscopic junctional conductance is as follows:

$$g(t) = \sum_{i=1}^{n} g_i(t) ,$$

where $g_i(t)$ – conductance of the *i*-th GJ channel at the time *t*, *n* – number of the channels. Conductance of GJ channel can be expressed through conductances of hemichannels in series, $g_i = g_{il} \cdot g_{ir} / (g_{il} + g_{ir})$. The model has to estimate conductance of each GJ channel at time moments, t_i ($i \in \{1, 2, \dots m\}$, $t_{i+1} = t_i + \Delta t$, $m\Delta t = T$).

2. Aggregate model

The model of gap junctions is composed from *n+2* aggregates as shown in Fig. 5. The aggregate '*Transjunctional voltage'* describes voltage loading to GJ channels. The aggregate '*GJ'* describes interaction between aggregate *Transjunctional voltage* and $C_1, C_2, \dots, C_n$ aggregates that represent a cluster of individual GJ channels. Output y_{n+1} of *GJ* aggregate is an averaged conductance of GJ channels. Aggregates $C_1, C_2, \dots, C_n$ describe changes of states and conductance of GJ channels over time.

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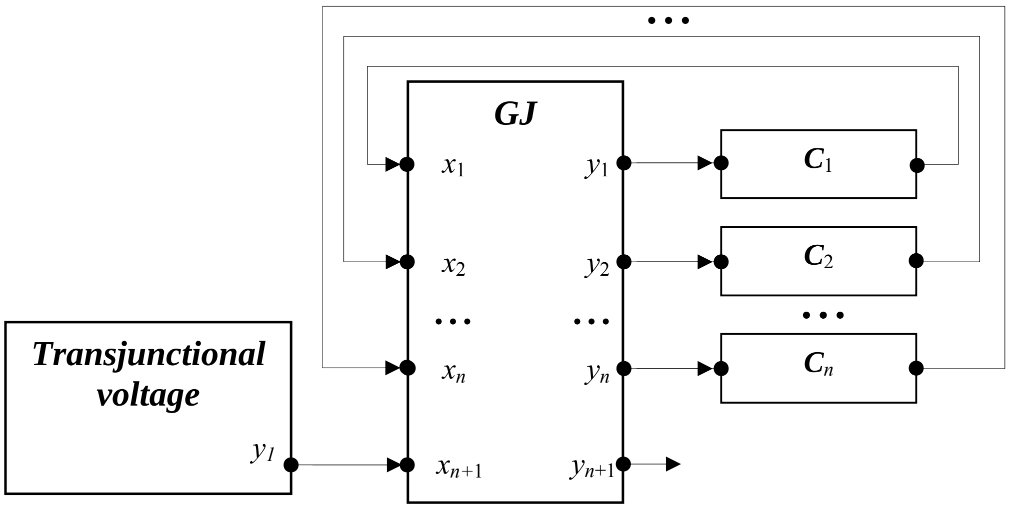

Fig. 5. Aggregate scheme of gap junctions.

Aggregate of *Transjunctional voltage*:

1. Input signals: $X = \varnothing$.
2. Output signals: $Y = \{y_1\}$, where y_1 – output voltage.
3. External events: $E' = \varnothing$.
4. Internal events: $E'' = \{e_1''\}$, where e_1'' – forms output voltage.
5. Controlling sequence, $e_1'' \mapsto \Delta\tau_1, \Delta\tau_2, \Delta\tau_3 \ldots$, where $\Delta\tau_i = \Delta\tau = const$ – discrete time interval that defines time instances of output signals.
6. Discrete state of the aggregate: $\nu(t_m) = \{U(t_m)\}$, where $U(t_m)$ – voltage supplied at t_m.
7. Continuous component of the aggregate state: $z_\nu(t_m) = \{w(e_1'', t_m)\}$, where $w(e_1'', t_m)$ indicate time instance when a new value of voltage is supplied to the channels.
8. Initial state: $t_0 = 0$, $z(t_0) = \{U(t_0), 0\}$.
9. Voltage loading function:

   $U(t): T \to R$.

10. Transition ($H(e_1'')$) and output ($G(e_1'')$) operators:

    $H(e_1'')$:

    $$\nu(t_{m+1}) = U(t_{m+1}),$$

    $$w(e_1'', t_{m+1}) = t_m + \Delta\tau.$$

    $G(e_1'')$:

    $$y_1 = U(t_{m+1}).$$

Aggregate *GJ*:

1. $X = \{x_1, x_2 \ldots x_n, x_{n+1}\}$, where $x_i = g_i(t_m)$– conductance of *i*-th channel and $i \in \{1,2,\ldots n\}$, $x_{n+1} = U(t_m)$– voltage across the channel.
2. $Y = \{y_1, y_2 \ldots y_n, y_{n+1}\}$, where y_i– the voltage across *i*-th channel and $i \in \{1,2,\ldots n\}$, y_{n+1}– an averaged conductance of all GJ channels.
3. $E' = \{e'_1, e'_2 \ldots e'_n, e'_{n+1}\}$, e'_i – external event related with x_i input.
4. $E'' = \varnothing$.
5. $\nu(t_m) = \{g_1(t_m), g_2(t_m), \ldots, g_n(t_m); \chi_1(t_m), \chi_2(t_m), \ldots, \chi_n(t_m)\}$, where $g_i(t_m)$– conductance of *i*-th channel; $\chi_i(t_m)$– indicator of *i*-th channel,
$$\chi_i(t_m) = \begin{cases} 0, & \text{aggregate has got information about conductance of } i\text{ - th channel,} \\ 1, & \text{aggregate is waiting information about conductance of } i\text{ - th channel.} \end{cases}$$
6. $z_\nu(t_m) = \varnothing$.
7. $\nu(t_0) = \{g_1(t_0), g_2(t_0), \ldots, g_n(t_0); \chi_1(t_0), \chi_2(t_0), \ldots, \chi_n(t_0)\}$.
8. Auxiliary variables $k(t_m), K$ and $g_{sum}(t_m)$ used for calculation of an averaged conductance of the channel, $k(t_0) = 0$, $g_{sum}(t_0) = 0$.
9. Transition ($H(e''_i)$) and output ($G(e''_i)$) operators:

$G(e'_{n+1})$:
$$y_1 = x_{n+1},\ y_2 = x_{n+1}, \ldots\ y_n = x_{n+1}.$$

$H(e'_i)$:
$$k(t_{m+1}) = \begin{cases} 1, & \sum_{k=1}^{n} \chi_k(t_{m+1}) = 0 \wedge k(t_m) = K, \\ k(t_m) + 1, & \sum_{k=1}^{n} \chi_k(t_{m+1}) = 0 \wedge k(t_m) < K, \end{cases}$$
$$\forall j \in \{1,2,\ldots n\}: \chi_j(t_{m+1}) = \begin{cases} 0, & i = j \wedge \sum_{k=1}^{n} \chi_k(t_m) < n-1, \\ \chi_j(t_m), & i \neq j \wedge \sum_{k=1}^{n} \chi_k(t_m) < n-1, \\ 1, & \sum_{k=1}^{n} \chi_k(t_m) = n-1, \end{cases}$$
$$\forall j \in \{1,2,\ldots n\}: g_j(t_{m+1}) = \begin{cases} x_i, & i = j, \\ g_j(t_m), & i \neq j, \end{cases}$$
$$g_{sum}(t_{m+1}) = \begin{cases} \frac{1}{n} \sum_{k=1}^{n} g_k(t_{m+1}), & k(t_{m+1}) = 1, \\ g_{sum}(t_m), & k(t_{m+1}) = k(t_m), \\ g_{sum}(t_m) + \frac{1}{n} \sum_{k=1}^{n} g_k(t_{m+1}), & k(t_{m+1}) \neq k(t_m). \end{cases}$$

$G(e'_i)$:
$$y_{n+1} = \frac{g_{sum}(t_{m+1})}{K}, \text{ if } k(t_m) = K.$$

Aggregate C_i, $i = 1,2,\ldots,n$:

1. $X = \{x_1\}$, where $x_1 = u(t_m)$– voltage across *i*-th channel.
2. $Y = \{y_1\}$, where y_1– channel conductance.
3. $E' = \{e'_1\}$, where e'_1– voltage across *i*-th channel.

4. $E'' = \{e_1''\}$, where e_1'' – event initiating a new state of *i*-th channel.
5. Controlling sequence $e_1'' \mapsto \Delta t_1, \Delta t_2, \Delta t_3 \ldots$, where $\Delta t_i = \Delta t = const$ – time interval during which a new state of the channel is calculated.
6. Discrete state of aggregate at time t_m: $v(t_m) = \{u(t_m), u_r(t_m), u_l(t_m), s_r(t_m), s_l(t_m), g_i(t_m)\}$, where $u(t_m)$– voltage across *i*-th channel, $u_r(t_m), u_l(t_m),$ – voltage across right and left gates, respectively, $s_r(t_m), s_l(t_m),$ – states of right and left gates, respectively, $g_i(t_m)$ – conductance of *i*-th channel.
7. Continuous component of the aggregate state: $z_v(t_m) = \{w(e_1'', t_m)\}$, where $w(e_1'', t_m)$ – time instance at which a new state of channel is determined and conductance is calculated.
8. $v(t_0) = \{u(t_0), u_r(t_m), u_l(t_m), s_r(t_0), s_l(t_0), g_i(t_0)\}$, $z_v(t_0) = \{\infty\}$.
9. Transition $(H(e_1'))$, ($H(e_1'')$) and output ($G(e_1'')$) operators:

$H(e_1')$:

$$w(e_1'', t_{m+1}) = t_m,$$
$$u(t_{m+1}) = x_1.$$

$H(e_1'')$:

$$s_r(t_{m+1}) = \begin{cases} o, & (s_r(t_m) = o \wedge \xi < p_{oo}^r(t_m)) \vee (s_r(t_m) = c \wedge \xi < p_{co}^r(t_m)), \\ c, & (s_r(t_m) = o \wedge p_{oo}^r(t_m) \le \xi \le 1) \vee (s_r(t_m) = c \wedge p_{co}^r(t_m) \le \xi \le 1), \end{cases}$$
$$s_l(t_{m+1}) = \begin{cases} o, & (s_l(t_m) = o \wedge \xi < p_{oo}^l(t_m)) \vee (s_l(t_m) = c \wedge \xi < p_{co}^l(t_m)), \\ c & (s_l(t_m) = o \wedge p_{oo}^l(t_m) \le \xi \le 1) \vee (s_l(t_m) = c \wedge p_{co}^l(t_m) \le \xi \le 1), \end{cases}$$
$$u_r(t_{m+1}) = \frac{u(t_{m+1}) g(t_m)}{g_r(t_m)},$$
$$u_l(t_{m+1}) = \frac{u(t_{m+1}) g(t_m)}{g_l(t_m)},$$
$$g_r(t_{m+1}) = f(s_r(t_{m+1}), v_r(t_{m+1})),$$
$$g_l(t_{m+1}) = f(s_l(t_{m+1}), v_l(t_{m+1})),$$
$$g(t_{m+1}) = \frac{g_r(t_{m+1}) + g_l(t_{m+1})}{g_l(t_{m+1}) \cdot g_r(t_{m+1})},$$
$$w(e_1'', t_{m+1}) = t_m + \Delta t.$$

$G(e_1'')$:

$$y_1 = g_i(t_{m+1}).$$

References:

1. Pranevicius H. 2005. Formal specification of computer network protocols: aggregate approach. Kaunas, Lithuania: “Technologija”. 188 p.
2. Packevicius S., A. Kazla, and H. Pranevicius. 2006. Extension of PLA specification for dynamic system formalization. Information Technology. N35, 235-242.