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Bifurcation Analysis of Regulation of Bursting Discharge in DRG Neurons

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Abstract. We examine the task of suppression of ectopic bursting discharges in dorsal root ganglion (DRG) of nociceptive neurons of rats. For solving the task we apply methods of bifurcation analysis of the DRG neuron model (Kovalsky et al. [13]) modified by including the TTX-resistant $Na_V1.8$ sodium channels in the system equations. It allows us to partition the parameter plane of the model into the regions corresponding to stable steady states, stable periodic solutions and bursting discharges and to find the system bifurcation parameters changing in which determine boundaries of the considered regions. We have shown that namely changes in parameters leading to modification of the $Na_V1.8$ sodium channels are responsible for switching off the ectopic bursting discharges. These changes cause a decrease in the effective charge transferred through the activation gating system of these channels in the membrane of the DRG neuron and reflect the specific action of comenic acid (5-hydroxy-γ-pyrone-2-carboxilic acid), which is a drug substance with a strong analgesic effect (Dick et al. [5]) Thus, our results explain the molecular mechanism of the analgesic suppression of ectopic bursting discharge in the nociceptive neuron.
Keywords: Bifurcation analysis, Fast and Slow variables, Bursting discharge, Suppression of neuropathic pain, DRG neuron model.

1 Introduction

It is known that pain sense modality is connected with activation of peripheral nociceptors recording signals and transmitting them by afferent nerve fibers to nociceptive neurons soma of which are in spinal ganglia. Low frequency of nerve impulses, as a rule, carries information about adequate tactile action while the rise of the frequency for amplification of the signal testifies about possible injury (Diss et al. [7]). Moreover, it is known that in response to injury of the nervous system nociceptive neurons can generate unusual ectopic bursting discharge (Amir et al. [1], Devor [3]).
It is assumed that bursts as units of neuronal information are more reliable than single spikes (Lisman [16]). First, they facilitate transmitter release. Second, they have higher signal-to-noise ratio than single spikes since their threshold exceeds the threshold of spikes (Sherman [20]). It is no wonder that bursting activity is likely to present during transmission of very important information such as pain sense modality.

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Slow sodium $Na_V1,8$ channels are considered to play the significant role in generation of painful feeling (Wu et al. [23]) since the enhancement of synthesis and functional activity of these channels is related to hyper-excitability of nociceptive neurons and the high frequency neuropathic pain emergence (Goldin [8], Lai et al [15]). The failure in the synthesis of the channels causes the relief of the pain (Waxman [21]). Modulation of activity of the channels by mediators of inflammation can lead to the pathological state such as hyperalgesia (that means an increase of the painful sensitivity) (Waxman et. al [22]). Hyperalgesia is removed by agents descending impulse activity of $Na_V1,8$ channels (Ogata and Ohishi [17]).
The aim of the work is to answer the question: what parameters of the slow sodium $Na_V1,8$ channels are responsible for switching off the ectopic bursting discharges and, hence, do maximal influence on pain signaling transduction?
To answer the question it is necessary to examine relations between these parameters, an applied external stimulus and a type of stable solution of the model system describing the impulse activity of the nociceptive neuron.

2 Model

It is known that the main contribution to the generation of pulse activity of the nociceptive DRG neuron under the action of stimulating current (I) comes from several sodium currents and the delayed potassium current (I_K) as well as the leakage current (I_L) (Kovalsky et al. [13]). Among the sodium currents one can isolate the fast activating and inactivating tetrodotoxin-sensitive current (I_{Naf}), the intermediate, fast activating and intermediately inactivating current (I_{NaI}) and the slow inactivating tetrodotoxin –resistant current (I_{NaS}). Based on these facts the model of the membrane of a nociceptive neuron can be described by the system of equations:

$$\begin{aligned} \frac{dE}{dt} &= (I - I_{Naf}(m,h,E) - I_{NaI}(b,E) - I_K(n,E) - I_L(E) - I_{Nas}(s,r,E))/c_m, \\ \frac{dx}{dt} &= (x_\infty(E) - x)/\tau_x(E), \qquad x = m,h,n,b,s,r \end{aligned} \tag{1}$$

where E is the membrane potential, I is the stimulating current. The ionic currents are determined by expressions:

$$\begin{aligned} I_{Naf} &= g_{Na} m^3 h(E - E_{Na}), \\ I_{NaI}(b,E) &= g_{NaI} m_{I\infty}(E) b(E - E_{Na}), \\ I_K(n,E) &= g_K n(E - E_K) \\ I_L(E) &= g_L(E - E_L), \\ I_{NaS}(s,r,E) &= g_{NaS} s^3 r(E - E_{Na}), \end{aligned}$$

where the variables m, h, n, b, s, r represent the gating characteristics of activation and inactivation of ionic channels, cm = 1 μF/cm^2 is the membrane

capacitance; $g_{Na} \in [0, 140]$ mS/cm^2, g_{NaI} = 27 mS/cm^2, g_K = 1.5 mS/cm^2, g_L = 1.4 mS/cm^2 , g_{NaS} = 5 mS/cm^2 are the maximal conductivities; and E_{Na} = 62 mV, E_K = –94 mV, E_L = –77 mV are the reversal potentials for Na^+, K^+ and leakage ions.

The potential-dependent steady-state values of the activation or inactivation variables corresponding to the population of all the ionic channels, except the Na_V1.8 sodium channels of the tetrodotoxin –resistant current, are defined by the functions given from the work devoted to the detailed description of the DRG neuron (Kovalsky et al. [13]):

$$m_\infty = 1./(1.+\exp(-(34.1+E)/9.1)),$$
$$h_\infty = 1./(1.+\exp((56.4+E)/7.2)),$$
$$m_{I\infty} = 1./(1.+\exp(-(25.3+E)/9.1)),$$
$$b_\infty = 1./(1.+\exp((72.5+E)/8)),$$
$$n_\infty = 1./(1.+\exp(-(9.2+E)/16)).$$

The time constants of these gating processes are described by the functions:

$$\tau_m = 0.01+0.11\exp(-0.5(((E+28.7)/25.5)^2)),$$
$$\tau_h = 0.24+1.63\exp(-0.5(((E+61.9)/15.3)^2)),$$
$$\tau_{m_I} = 0,$$
$$\tau_b = 0.22\exp(-0.07E),$$
$$\tau_n = -23.+69.4\exp(-0.01E).$$

The potential-dependent steady-state values of the activation or inactivation variables of the tetrodotoxin –resistant sodium channels and their time constants are defined as

$$x_\infty(E) = \alpha_x(E)/(\alpha_x(E)+\beta_x(E)),$$
$$\tau_x(E) = 1./(\alpha_x(E)+\beta_x(E)), \qquad x = s, r$$

where the potential-dependent rate constants for transitions of the activation and inactivation gating structures of the channels between the closed and open states were determined using the multiparametric least square method in our previous work (Dick et al. [6]). The minimization of the functional was performed by the gradient minimization method for the best agreement with the experimental data obtained under voltage clamp conditions.

Under the control conditions, these dependences for the activation gating system of the tetrodotoxin –resistant sodium channel are found as

$$\alpha_s = \exp(a_1E+b_1) = \exp(0.043E-2.22),$$
$$\beta_s = \exp(a_2E+b_2) = \exp(-0.048E-4.33)$$

After treatment with comenic acid (5-hydroxy-γ-pyrone-2-carboxilic acid) in a concentration of 100 nmol/L, these dependences are written in the form

$$\alpha_s = \exp(0.047E-2.71),$$
$$\beta_s = \exp(-0.015E-4.05)$$

According to the Boltzmann's principle for the channel with the two-state open-closed structure, the ratio of the number of open channels (N_0) to the number of closed channels (N_C) is determined by

$$N_o / N_c = s/(1-s) = \exp(Z_{eff}\bar{e}(E-\bar{E})/kT),$$

where Z_{eff} is the effective charge of the activation gating structure (in electron units) coupled with conformational change of the gating structure during the ion transfer through the membrane, k is the Boltzmann's constant, T is the absolute temperature, $\bar{e}$ is the electron charge, $\bar{E}$ is the membrane potential such that $N_0=N_C$

Then at $E=\bar{E}$ for the activation gating structure of the slow sodium channels one can write $\alpha_s = \beta_s$, whence it follows that the effective charge value of the activation gating structure (in the electron charge units) can be gained as

$$Z_{eff} = (3kT/e)(a_1 - a_2),$$

where coefficient 3 takes into account the fact that the behavior of the activation gating system of sodium channels corresponds to the three - barrier model.

Changes in the dependences for the activation gating system of the tetrodotoxin –resistant sodium channel after treatment with comenic acid correspond to a decrease in the effective charge from the value of Z_{eff} = 6.9 obtained in control experiments to the value of Z_{eff} = 4.7 obtained after the action of comenic acid.

3. Partition of the model parameter space into regions of qualitatively different solutions

To obtain relationship between the type of stable solution of the system, its parameters and an applied external stimulus it is sufficient to find points belonging to the boundary partitioning the parameter space of the model system into the regions of the qualitatively different types of stable solutions (steady states and stable periodic oscillations). The method of bifurcation analysis is applied for constructing the boundary.

It is known that for the Hodgkin-Huxley type system there are at least 3 bifurcation points ($I_0<I_1<I_2$) on I axis (Hassard [11]). For $I<I_0$ and $I>I_1$ there is a one-to-one correspondence between the type of steady state (unstable or stable) and the presence or absence of a stable periodic solution. For $I \leq I_0$ and $I \geq I_2$ the steady state is stable and a limit cycle does not exist.

In interval ($I_0<I<I_1$) there is a bistability connected with the coexistence of the stable steady state and a pair of stable and unstable limit cycles appearing via fold limit cycle bifurcation (a saddle-node bifurcation of limit cycles) (Rinzel and Miller [19]). Then the unstable limit cycle shrinks down to the rest state. At I_1 the steady state loses stability via a subcritical Andronov-Hopf bifurcation resulting in an unstable limit cycle of large amplitude.

Since for the Hodgkin-Huxley type system $I_0 \approx I_1$ for all the physiologically possible parameter values (Bedrov et al. [2]), the value of I_1 can be used as an approximate value of I_0. Therefore, the task of finding the boundary of

qualitatively different types of stable solutions can be reduced to the more simple numerical task of constructing the boundary of various steady states (stable and unstable).

We write system (1) in the form

$$dy/dt = F(y, p, I), \tag{2}$$

where y=(E, m, h, n, b, s, r) is a vector of the phase coordinates, p is a vector of parameters which can be considered as bifurcation ones. The search of the boundary points of the region of stable periodic solutions is reduced to the sequence of procedures:

1) finding the equilibrium state of system (2) as a solution y_0 (p, I) of the equation

$$F(y, p, I)=0$$

2) calculating the eigenvalues $\{\lambda_i(p,I)\}_1^7$ of the Jacobian matrix

$$J(p,I) = \left(\partial F_i / \partial y_j \Big|_{y=y_{0(p,I)}}, i, j = 1,\ldots 7\right)$$

3) finding the parameter values satisfying the Andronov-Hopf bifurcation, namely, arising of a pair of complex-conjugate eigenvalues with zero real part:

$$\lambda_1 = i\omega, \quad \lambda_2 = -i\omega, \quad \lambda_3 < 0, \quad \lambda_4 < 0, \quad \lambda_5 < 0, \quad \lambda_6 < 0, \lambda_7 < 0$$

(Kuznetsov [14]).

The fold (saddle-node) bifurcation occurs when the Jacobian matrix at the equilibrium has a zero eigenvalue (Kuznetsov [14]).

The bifurcation analysis with dividing the parameter plane (I, p) into the regions of different solutions was performed using the software package MATCONT (Dhooge et al. [4]). The numerical solution of system (1) inside the obtained region of stable periodic solutions was found by a fourth-order Runge-Kutta method with a modified variable step size and Gear algorithm with the minimal step size and tolerance of integration set as 10^{-14} and 10^{-9} correspondingly.

To find the boundaries of bursting activity of the model the slow - fast decomposition of the initial system was applied. Such decomposition is necessary due to the transitions between quiescent and active states in bursting rhythms are marked by bifurcations of the fast subsystem (Guckenheimer et al. [9]).

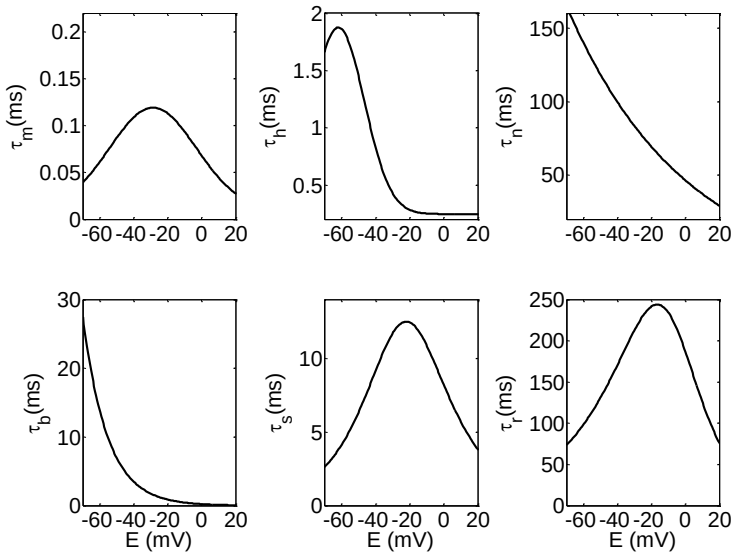

Fig. 1 The time constants of the gating characteristics of the model (1).

The decomposition of the full system into the slow and fast subsystems is based on the fact that the system has multiple time scales (Rinzel and Lee [18]). As seen in Fig.1 the channels of the delayed potassium current and the slow inactivating tetrodotoxin –resistant sodium current have the time constants the values of which (τ_n and τ_r) are considerably greater than the others. It enables us to consider the fast subsystem in the form

$$\frac{dE}{dt} = (I - I_{Naf}(m,h,E) - I_{NaI}(b,E) - I_K(n,E) - I_L(E) - I_{Nas}(s,r,E))/c_m,$$

$$\frac{dx}{dt} = (x_\infty(E) - x)/\tau_x(E), \qquad x = m,h,b,s,$$

where the slow variables *n* and *r* are considered to be parameters. Therefore, the slow – fast decomposition reduces the task of analysis the full system to the bifurcation problem of the fast subsystem with slow-varying bifurcation parameters *n* and *r*.

The slow system then can be represented as

$$I - I_{Naf}(m,h,E) - I_{NaI}(b,E) - I_K(n,E) - I_L(E) - I_{Nas}(s,r,E) = 0,$$

$$\frac{dx}{dt} = (x_\infty(E) - x)/\tau_x(E), \qquad x = n,r$$

The onset of the burst's active phase typically corresponds to a loss of fixed point stability in the fast system, and the termination of the active phase

corresponds to a loss of limit cycle stability in the fast system (Guckenheimer and Holmes [10]).

4. Results and discussion

The boundaries partitioning the parameter plane (g_{Na}, I) into the regions of stable and unstable steady states of the full system are given in Fig.2.

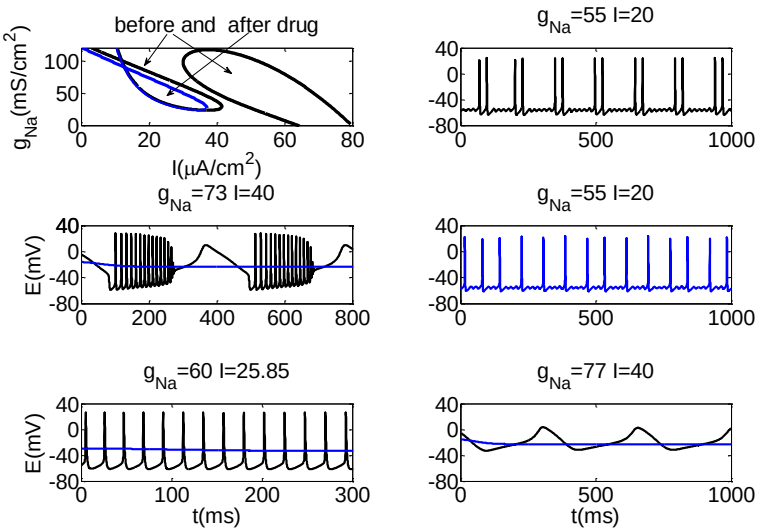

Fig. 2. The boundaries partitioning the parameter plane (g_{Na}, I) into the regions of stable and unstable steady states of the model (1) before and after modification of the tetrodotoxin –resistant sodium channels.
The examples of steady solutions inside the regions for different values of parameters g_{Na} .and I (black and blue curves correspond to the states before and after the modification, respectively).

The boundaries constructed are connected with the Andronov-Hopf bifurcation of the full system. Inside the each found region the steady state is unstable and there is a stable limit cycle corresponding to stable periodic solution. Examples of these solutions are shown in Fig. 2. On the parameter plane (g_{Na}, I) the initial full system has two regions, the boundaries of which are labeled by the black curves. The right region disappears in the modified full system. Moreover, modification of parameters of the activation gating system of the tetrodotoxin – resistant $Na_V1.8$ sodium channels caused by the action of comenic acid (5-hydroxy-γ-pyrone-2-carboxilic acid) results in either suppression of ectopic bursting discharges or even switching off the impulse activity in accordance with the values of sodium channel density and stimulus applied. Thus, we have shown that the drug substance with a strong analgesic effect (Dick et al. [5]) can exclude the ectopic discharge of nociceptive neurons.

Modification of the parameters after treatment with comenic acid are connected with a decrease in the effective charge coupled with conformational change of the activation gating structure during the ion transfer through the membrane.
This value changes from Z_{eff} = 6.9 obtained in control experiments to the value of Z_{eff} = 4.7 obtained after the action of comenic acid.
Therefore, we have proved that parameters of the slow sodium $Na_V1,8$ channels are responsible for switching off the ectopic bursting discharges and, hence, do maximal influence on pain signaling transduction.
Fig. 3 illustrates one of the possible mechanisms of the bursting emergence.

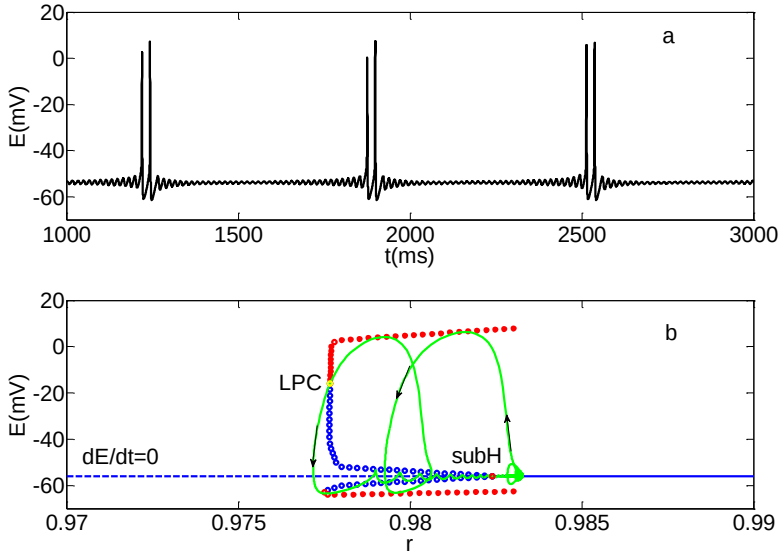

Fig. 3. The example of a subHopf /fold cycle burster of the model (1) before modification of the tetrodotoxin –resistant sodium channels for the parameters
g_{Na} = 39.71mS/cm^2, I = 22.4 μA/cm^2.
The trajectory of the full system (green curve) is plotted in projection on the (r, E) plane, along with the bifurcation diagram of the fast system. The bifurcation diagram includes the branch of fixed points and periodic orbits. Solid/dashed blue curves indicate stable/unstable points (steady states). Unstable orbits are labeled by blue open circles and stable orbits depicted by red closed circles, indicating maximal and minimal values of E over the orbit.

On the one – parameter bifurcation diagram represented in Fig.3 b the value r is considered as a bifurcation parameter. During the quiescent state of the burst the trajectory of the full system decreases in r value along the branch of fixed points of the fast system.
The active phase of the burst initiates when the trajectory passes through the subH point. Hence, the quiescent state loses stability via subcritical Andronov-Hopf bifurcation of the fast subsystem. The trajectory of the full system ramps

from this point, oscillating with growing amplitude until it reaches the stable periodic branch of the large amplitude. Then the trajectory moves leftwards until it reaches the fold limit cycle bifurcation point (LPC point). Finally, the trajectory of the full system returns to the *E*-nullcline (dE/dt=0) with a few damped oscillations.

Thus, this is the subHopf /fold cycle burster by classification (Izhikevich [12]) since the active phase of bursting begins at a subcritical Hopf bifurcation point and ends at a fold limit cycle bifurcation point of the fast system.

Fig. 4 illustrates the other possible mechanism of the bursting emergence.

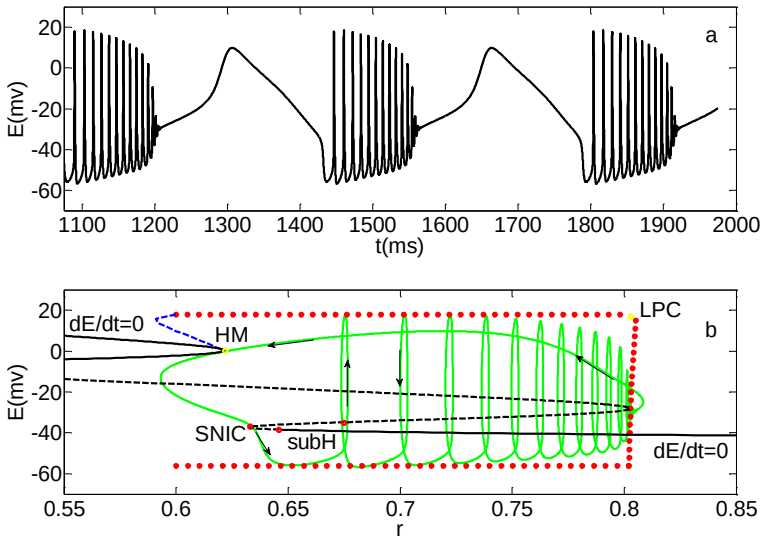

Fig. 4. The example of a circle/fold cycle via homoclinic/circle hysteresis loop (cycle-cycle burster) of the model (1) before modification of the tetrodotoxin –resistant sodium channels for the parameters g_{Na} = 63.59 mS/cm^2, I = 44.3 μA/cm^2.

The active phase of the burst initiates when the trajectory passes through the saddle-node bifurcation on invariant circle (SNIC point which coincides with the saddle–node of fixed points and situated near the subcritical Hopf bifurcation point (subH)) of the fast subsystem. Such bifurcation results in appearance of a stable limit cycle of large amplitude. Then the trajectory of the full system oscillates with increasing frequency and moves rightwards until it reaches the fold limit cycle bifurcation point (LPC point). Finally, the trajectory of the full system touches the *E*-nullcline (dE/dt=0) at a saddle-node homoclinic bifurcation resulting in the birth of another limit cycle with the period tending to infinity. Then the full trajectory reaches the SNIC point again and the bursting

repeats. Thus, this is the circle/fold cycle via homoclinic/circle hysteresis loop (cycle-cycle burster) by classification (Izhikevich [12]).

Conclusions

The bifurcation analysis of regulation of impulse activity in DRG neurons enables us to answer positively the question: what parameters of the slow sodium $Na_V1,8$ channels are responsible for switching off the ectopic bursting discharges? The obtained results allow us to explain also the molecular mechanism of the analgesic suppression of bursting discharge in the nociceptive neurons.

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