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Afterhyperpolarization improves spike programming through lowering threshold potentials and refractory periods mediated by voltage-gated sodium channels ☆

Na Chen, Xin Chen, Jiandong Yu, Jinhui Wang *

State Key Lab for Brain and Cognitive Sciences, National Lab for Protein Sciences, Institute of Biophysics Chinese Academy of Sciences, Beijing 100101, China

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Abstract

Neurons program various patterns of sequential spikes as neural codes to guide animal behavior. Studies show that spike programming (capacity and timing precision) is influenced by inhibitory synaptic inputs and membrane afterhyperpolarization (AHP). Less is clear about how these inhibitory components regulate spike programming, which we investigated at the cortical neurons. Whole-cell current-clamp recording for action potentials and single channel recording for voltage-gated sodium channels (VGSC) were conducted at regular-spiking and fast-spiking neurons in the cortical slices. With quantifying the threshold potentials and refractory periods of *sequential spikes*, we found that fast-spiking neurons expressing AHP possess lower threshold potentials and shorter refractory periods, and the hyperpolarization pulse immediately after each of spikes lowers threshold potentials and shortens refractory periods at regular-spiking neurons. Moreover, the hyperpolarization pulses shorten the refractory periods for VGSC reactivation and threshold potentials for its sequential activation. Our data indicate that inhibitory components immediately after spikes, such as AHP and recurrent inhibition, improve spike capacity and timing precision via lowering the refractory periods and threshold potentials mediated by voltage-gated sodium channels.
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Keywords: Action potential; Spike timing precision; Spike capacity; Refractory period; Threshold potential; Hyperpolarization and neurons

The precise behaviors (e.g., perception, motion, and cognition) are controlled by the meaningful signals programmed at neurons and synapses under the physiological conditions. The molecular bases of such behaviors were studied [1,2]. Less is known about how the neurons program the neural codes precisely. Sequential spikes, likely the digital signals from silicon-based switch, are thought of the preferred candidates for neural computations [3–8]. Spike timing is also critical [9,10] since precise and loyal spike patterns signify neuronal events in meaningful and memorable manner. It is pivotal to uncover mechanisms navigating spike timing precision and capacity.

The spike patterns are believed to be modulated by synapse dynamics [6,7,11,12], and by membrane potentials in experiments [4,13–16] or in simulation model [17–19]. Afterhyperpolarization (AHP) generated from the potassium channels and recurrent inhibitory synapses affects neuronal excitability as well as spike timing [20–26]. We found that threshold potentials and refractory periods control spike programming [27]. Do membrane potentials and synapse dynamics influence the programming of sequential spikes in direct manner or via altering the refractory periods and threshold potentials mediated by voltage-gated sodium channel (VGSC)?

☆ *Abbreviations:* Vr, resting membrane potential; Vm, membrane potential; Vts, threshold potential; Vts-Vr, the difference between threshold potential and resting membrane potential; RP, refractory period; AHP, afterhyperpolarization; RSN, regular-spiking neuron; FSN, fast-spiking neuron; ISI, inter-spike interval; SDST, standard deviation of spike timing; VGSC, voltage-gated sodium channels.

* Corresponding author. Fax: +86 10 64888472.
E-mail address: jhw@sun5.ibp.ac.cn (J. Wang).

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To questions above, we investigated the influences of afterhyperpolarization on sequential spikes as well as their threshold potentials and refractory periods at the fast-spiking neurons (FSN) with expressing AHP and regular-spiking neurons (RSN) without AHP in cortical slices by whole-cell clamp recordings. We also examined such influences by recording the activities of single VGSCs.

Materials and methods

Brain slices. Cortical slices (400 μm) were prepared from Sprague-Dawley rats (postnatal day16–22) that were anesthetized by injecting pentobarbital (50 mg/kg) and decapitated with a guillotine. The slices were cut with a Vibratome in the modified and oxygenized (95% O_2/5% CO_2) artificial cerebrospinal fluid (mM: 124 NaCl, 3 KCl, 1.2 NaH_2PO_4, 26 $NaHCO_3$, 0.5 $CaCl_2$, 5 $MgSO_4$, 10 dextrose, and 5 Hepes, pH 7.35) at 4 °C, and then were held in the normal oxygenated ACSF (mM: 124 NaCl, 3 KCl, 1.2 NaH_2PO_4, 26 $NaHCO_3$, 2.4 $CaCl_2$, 1.3 $MgSO_4$, 10 dextrose, and 5 Hepes, pH 7.35) at 24 °C for 1–2 h before experiments. A slice was transferred to the submersion chamber (Warner RC-26G) that was perfused with the normal ACSF at 31 °C for the whole-cell recordings [27]. Chemicals were purchased from Fisher Scientific. The procedures were approved by IACUC in Beijing, China.

Neuron selection. Neurons in the layer II–III of sensorimotor cortex were recorded. Regular-spiking neurons (RSN) show pyramidal-like soma and an apical dendrite; and fast-spiking neurons (FSN) are round with multiple processes under DIC optics (Nikon FN-E600). RSN and FSN show different properties in the response to current pulses [27–29].

Whole-cell recording. Action potentials were recorded with multi-clamp 700B, and inputted into pClamp 9 (Axon Instrument Inc., Foster CA, USA) for data acquisition and analyses. Output bandwidth was set at 3 kHz. Spike patterns and modulation at RSN and FSN were studied by using the simulated pulses or currents (depolarization, hyperpolarization or mixed pulses). The intensity of currents for evoking sequential spikes equals the threshold pulse (10 ms) of eliciting a single spike. The standard pipette solution contains (mM) 150 K-gluconate, 5 NaCl, 10 Hepes, 0.4 EGTA, 4 Mg-ATP, 0.5 Tris–GTP, and 4 Na-phosphocreatine (pH 7.4, adjusted by 2 M KOH). Fresh pipette solution was filtered with 0.1 μm centrifuge filter. Its osmolarity was 295–305 mOs mol; and pipette resistance was 6–8 MΩ.

Neuronal intrinsic properties. The threshold potentials and refractory periods of sequential spikes were measured and defined in previous study [27]. Inter-spike intervals (ISI, an index of spike capacity) are the duration between a pair of spikes; and the standard deviation of spike timing (SDST, an index of spike precision) is the standard deviation of spike lock-phase. Data were analyzed if Vr was above −60 mV at the recorded FSN and −67 mV at RSN. The criteria for the acceptation of each experiment also included less than 5% changes in Vr, spike magnitude, and input resistance throughout each of experiments [27]. The values of Vts, RP, ISI, and SDST are presented as means ± SE. The comparisons between a pair of treatments were done by *t*-test.

Single-channel recording. The signals from VGSC were recorded in cell-attached configuration with multi-clamp 700B and pClamp-9 at neurons in cortical slices. Seal resistance was above 20 GΩ, and pipette resistance was 10–15 MΩ. Pipette solution contains (mM) 120 NaCl, 2 MgCl2, 10 Hepes, 30 TEA, and 0.1 mibefradil. TEA and mibefradil were used to block voltage-gated potassium and type-T calcium channels, respectively. Threshold potentials for VGSC activation were measured when adding negative voltage pulses (4 ms) into the recording pipettes; and refractory periods for VGSC reactivation were measured when changing inter-pulse intervals (IPI) in 6–8 ms. We also measured ARP for VGSC reactivation by raising depolarization intensity. The events of single VGSCs in Fig. 5 are summated.

Results

The strategy for addressing the mechanisms underlying the influences of AHP on spike capacity and timing precision was comparing the influences of the given inputs on spike patterns and intrinsic properties at the neurons with and without expressing AHP. This approach is based on a principle of occlusion that spike programming at the neurons expressing natural AHP should not be affected significantly by a given AHP.

Spike programming at neurons with and without AHP

The current pulses integrated from hundreds of excitatory synapses were injected into RSN and FSN to elicit sequential spikes. Fig. 1 shows the influences of excitatory inputs on inter-spike intervals (ISI) and standard deviation of spike timing (SDST). To the given inputs (260 ms) at threshold intensity that was the value of 5 ms pulse for a single spike, the number and timing of spikes are better at FSN than RSN. The values for $SDST_1$ to $SDST_4$ are 1.45 ± 0.2, 2.24 ± 0.26, 3.39 ± 0.5, and 5.13 ± 0.74 ms at FSN (circles, $n = 17$); the values are 1.12 ± 0.29, 3.58 ± 0.54, 5.46 ± 0.73, and 8.29 ± 0.94 ms at RSN (triangles, $n = 15$). The values from ISI_{1-2} to ISI_{4-5} are 35.72 ± 3.6, 41.1 ± 4.56, 47.56 ± 4.7, and 51.56 ± 4.84 ms at FSN (circles); the values are 38.57 ± 2.2, 53.89 ± 2.97, 64.34 ± 2.56, and 77.54 ± 3.84 ms at RSN (triangles). ISI and SDST relevant to the same number of sequential spikes are statistically lower at FSN than RSN ($p < 0.01$), except for spike one. These data indicate that FSN has higher ability in the programming of sequential spikes compared to RSN.

FSN expresses AHP (Fig. 1 and Ref. [28,30,31]). AHP mediated by the potassium channels and/or inhibitory synapses presumably influences the neuronal excitability and spike timing [6,20–26]. The data imply that AHP raises neuronal ability in programming spikes.

AHP improves spike capacity and timing precision

To address the role of inhibitory components in regulating spike programming, we injected depolarization pulses to evoke spikes and HP pulses (3 ms) immediately after each of spikes to simulate AHP. If HP pulses improve spike programming at RSN with no AHP, but not FSN with AHP, we are able to conclude that AHP is a critical factor in the improvement of spike programming. Fig. 2 shows the expression of spike capacity and timing precision at RSN (A–C) and FSN (D–F) without and with giving HP pulses.

Short-term HP pulses strengthen spike programming at RSN. Fig. 2B and C illustrates the quantitative analyses of ISI between spike one and two (ISI_{1-2}) up to four and five (ISI_{4-5}) as well as of $SDST_1$ to $SDST_5$. The values from ISI_{1-2} up to ISI_{4-5} are 17.2 ± 1, 31.5 ± 1.24, 37.87 ± 1.23, and 42.46 ± 1.42 ms under controls (opened triangles);

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