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Frequency-dependent regulation of intrinsic excitability by voltage-activated membrane conductances, computational modeling and dynamic clamp

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Abstract

As one of the most unique properties of nerve cells, their intrinsic excitability allows them to transform synaptic inputs into action potentials. This process reflects a complex interplay between the synaptic inputs and the voltage-dependent membrane currents of the postsynaptic neuron. While neurons in natural conditions mostly fire under the action of intense synaptic bombardment and receive fluctuating patterns of excitation and inhibition, conventional techniques to characterize intrinsic excitability mainly utilize static means of stimulation. Recently we have shown that voltage-gated membrane currents regulate the firing responses under current step stimulation and under physiologically more realistic inputs in a differential manner. At the same time, a multitude of neuron types have been shown to exhibit some form of subthreshold resonance that potentially allows them to respond to synaptic inputs in a frequency-selective manner. In the present study we performed virtual experiments in computational models of neurons to examine how specific voltage-gated currents regulate their excitability under simulated frequency-modulated synaptic inputs. The model simulations and subsequent dynamic clamp experiments on mouse hippocampal pyramidal neurons revealed that the impact of voltage-gated currents in regulating the firing output is strongly frequency-dependent and mostly affecting the synaptic integration at theta-frequencies. Notably, robust frequency-dependent regulation of intrinsic excitability was observed even when conventional analysis of membrane impedance suggested no such tendency. Consequently, plastic or homeostatic regulation of intrinsic membrane properties can tune the frequency-selectivity of neuron populations in a way that is not readily expected from subthreshold impedance measurements.

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Graphical abstract

We assessed the role of various voltage-gated currents in regulating the firing responses of three types of model neurons under frequency-modulated synaptic inputs. Up- or downregulation of the intrinsic membrane currents regulated the firing responses in a strongly frequency-dependent manner that was not readily expected from analysis of the subthreshold resonance properties of the model neurons. In agreement with the model predictions, we observed robust, frequency-dependent regulation of excitability of hippocampal pyramidal neurons under the insertion of a computer-synthesized inward rectifying K-current in dynamic clamp experiments.

Keywords

resonance; computational model; physiological properties; oscillation; firing

Introduction

Each action potential emitted during a lifetime of a neuron is produced by a complex interplay between the synaptic inputs and intrinsic cellular properties. Voltage-gated ionic channels play a central role in the generation and timing of spikes and a great deal of effort has been made to elucidate how each current contributes to the regulation of firing responses. Conventional experimental procedures addressing the neurons' intrinsic excitability involve intracellular stimulation using constant levels of current providing the input-output relationships of neurons. The merit of static stimulation and the interpretation of data from such experiments have been rarely questioned, although neurons in natural conditions typically operate in high conductance state and under heavy synaptic bombardment (Destexhe *et al.*, 2003). As an example, if downregulation or blocking of an intrinsic K-current doubled the firing rate of a neuron under current step stimulation, would that produce the same effect under real synaptic inputs? To examine this scenario, we have recently performed biological experiments and computational modeling on neurons of the rodent extended amygdala. We have found that static and dynamic excitability in such conditions were regulated by voltage-gated membrane currents in a differential manner (Szűcs & Huerta, 2015).

As one of the more intricate dynamical properties of neurons, electrical resonance has been demonstrated in several neuronal phenotypes and involving several ionic currents (Hutcheon & Yarom, 2000) (Hutcheon *et al.*, 1996b; Hu *et al.*, 2002; Peters *et al.*, 2005; Hsiao *et al.*,

2009). Resonant properties have been suggested to mediate frequency selective integration of synaptic inputs (Schreiber *et al.*, 2004; Beatty *et al.*, 2015) and promote the generation of circuit oscillations (Pape & Driesang, 1998; Stark *et al.*, 2013; Schmidt *et al.*, 2016). Nevertheless, it is less clear whether findings on subthreshold membrane resonance also extend to the integration of random or quasiperiodic fluctuating synaptic inputs (Haas & White, 2002; Schreiber *et al.*, 2004; Kispersky *et al.*, 2012). Our present study addresses the question how specific voltage-gated membrane currents regulate the intrinsic excitability of neurons under the action of oscillatory synaptic inputs. In particular, we analyze firing responses of computational models of three biophysically distinct neuron types using conventional current stimulation as well as under simulated synaptic bombardment. By manipulating the maximal conductances of selected voltage-gated currents we assess their role in regulating the firing output. We find that the majority of the intrinsic currents promote resonant type excitability profiles, i.e. robust, frequency-dependent regulation of their firing output even when analysis of their subthreshold membrane impedance indicates no tendency for such behavior. Finally, we use the dynamic clamp technique to simulate oscillatory synaptic inputs and to insert computer-generated voltage-dependent currents into mouse hippocampal pyramidal neurons. We find that the artificial upregulation of the inward rectifying K-current in the biological neurons reduces their excitability most effectively at theta-frequencies of the oscillatory synaptic drive in good agreement with the model predictions.

Materials and methods

Single neuron models and synaptic input

We designed three model neurons each consisting of a dendritic, a somatic and an axonic compartment (passive membrane properties are listed in Table 1.). All intrinsic voltage-dependent currents were calculated as

$$I_i = g_i m_i^p h_i (E_i - V),$$

where i represents the individual current type, g_i is the maximal conductance of the current, m_i is the activation variable, p is the exponent of the activation term, h_i is the inactivation variable (either first-order or absent) and E_i is the reversal potential. Differential equations for the activation (m) and inactivation (h) share the same form (x being either m or h):

$$\frac{dx}{dt} = \frac{x_\infty(V) - x}{\tau_x(V)},$$

where voltage-dependent steady-state activation and inactivation are described by sigmoids:

$$x_\infty(V) = \frac{1}{2} + \frac{1}{2} \tanh \left(\frac{V - V_{x,1/2}}{V_{x,sl}} \right).$$

The midpoint $V_{x,1/2}$ and slope $V_{x,sl}$ parameters of the sigmoids and the other kinetic parameters are shown in Table 2. Time constant of the activation and inactivation are bell-shaped functions of the membrane potential:

$$\tau_x(V) = (\tau_{x,max} - \tau_{x,min}) \left[1 - \tanh \left(\frac{V - V_{\tau x,1/2}}{V_{\tau x,sl}} \right)^2 \right] + \tau_{x,min}.$$

The Ca-dependent K-current and internal Ca-dynamics were based on the formalism in (Canavier & Landry, 2006). Synaptic currents were described using a first-order kinetics of transmitter release (Pinto *et al.*, 2001) as:

$$I_{syn} = g_{syn} S (E_{syn} - V),$$

where S is the instantaneous synaptic activation term yielding the following differential equation:

$$\frac{dS}{dt} = \frac{S_{\infty}(V_{pre}) - S}{\tau_{syn}(1 - S_{\infty}(V_{pre}))}.$$

The steady-state synaptic activation term depends on the presynaptic membrane potential as

$$S_{\infty}(V_{pre}) = \tanh \left(\frac{V_{pre} - V_{th}}{V_{slope}} \right),$$

when $V_{pre} > V_{th}$, otherwise $S_{\infty}(V_{pre}) = 0$. V_{pre} denotes the presynaptic membrane potential waveform that is stored in ASCII files and designed prior to the model runs (Szűcs *et al.*, 2010). The reversal potential of the excitatory and inhibitory synaptic connections was 0 and -72 mV, respectively. Other synaptic parameters were as follows: $\tau_{syn} = 10$ ms; $V_{th} = -40$ mV; $V_{slope} = 20$ mV.

Protocols to characterize intrinsic excitability and subthreshold resonance

We used two types of stimulation to elicit firing responses of the model neurons and to evaluate their static and dynamic excitability. The first protocol was based on somatic injection of gradually more depolarizing current (500 ms steps, 2 pA increment; Fig. 1). The minimal current eliciting an action potential (rheobase) was determined and stimulation was continued until reaching 2-times rheobase level. Cumulative spike number was obtained by counting all spikes fired during this protocol. The second type of stimulation was designed to elicit firing responses under simulated synaptic inputs. Concurrent excitation and inhibition via AMPA- and GABA-type synaptic connections were set up using independent voltage waveforms to simulate excitatory and inhibitory presynaptic neurons (Fig. 4A and

B). To obtain dynamic I-O functions of the model neurons we incremented the synaptic conductances of both the AMPA- and GABA-inputs in successive sweeps of stimulation. Stimulation was continued until 2-times the threshold conductance that elicited the first suprathreshold response.

Subthreshold resonance of the model cells was studied by somatic injection of a frequency-modulated sinusoidal current waveform commonly referred to as ZAP protocol (Hutcheon *et al.*, 1996a). The frequency of the waveform was swept from 0 to 30 Hz under 65 s. The amplitude of the current was set individually for each neuron model to a level 0.1 pA below the observed firing threshold. Membrane impedance was defined as the ratio of the absolute magnitudes of the FFT of the voltage response and the current input:

$$Z = \frac{\text{FFT}(V_m)}{\text{FFT}(I_{\text{ZAP}})},$$

Dynamic clamp experiments on cultured hippocampal neurons

Primary cultures of embryonic hippocampal cells were prepared from CD1 mice on embryonic day 17-18 according to (Czöndör *et al.*, 2009). Cells were seeded onto poly-L-lysine and laminin coated glass coverslips in 24-well plates at $1.2\text{--}1.3 \times 10^5$ cells/well density. Electrophysiological recordings were performed in whole-cell conditions at room temperature (21-23 °C) using a MultiClamp 700B amplifier (Molecular Devices). Stimulus waveforms serving as presynaptic voltage for the dynamic clamp system were generated by the data acquisition software DASyLab v. 11 (National Instruments). The same program was used to record the voltage responses of the hippocampal neurons at 20 kHz sampling rate. The composition of the bath solution (ACSF) was (in mM): NaCl 140, KCl 5, CaCl₂ 2, MgCl₂ 1, HEPES 5, D-glucose 10; pH 7.45. For recording membrane potential and to inject the dynamic clamp-generated current single whole-cell patch electrodes were used (6-8 MOhm) and filled with the following solution (in mM): K-gluconate 100, KCl 10, KOH 10, MgCl₂ 2, NaCl 2, HEPES 10, EGTA 0.2, D-glucose 5; pH set to 7.3. For dynamic clamp experiments we used the StdPC v. 2012 program allowing concurrent simulation of chemical synaptic connections and insertion of Hodgkin-Huxley-type voltage-gated currents (Nowotny *et al.*, 2006). Simulated synaptic inputs to the biological neurons were generated as described in (Szücs & Huerta, 2015). We used simulated chemical synaptic connections to couple one excitatory (AMPA-) and one inhibitory (GABA-) input to the biological neuron. The synaptic time constant was 10 ms for both the AMPA- and GABA-connections, reversal potential was 0 mV and -72 mV, respectively. The excitatory synaptic conductance was incremented by 2 nS in the successive sweeps of the stimulation while the increment of the inhibitory conductance was 4 nS. Additionally, a synthetic inward rectifying K-current was generated by the dynamic clamp. Parameters of voltage dependence and kinetics of this current were as in the model simulations (see Table 2). Our dynamic clamp system allowed the insertion of the synthetic K_{ir}-conductance during the concurrent stimulation with current steps or simulated synaptic bombardment.

Results

Construction of models of neuronal phenotypes

We designed conductance-based models of three types neurons exhibiting distinct biophysical properties. Two of the cell types were based on our prior electrophysiological experiments and additional voltage clamp data of neurons of the bed nucleus of stria terminalis (BNST). Earlier studies, including ours, identified 3 phenotypes of GABAergic neurons in the BNST (Hammack *et al.*, 2007; Szücs *et al.*, 2012). Of the three types, type II and type III neurons exhibit markedly different physiological and biophysical properties. Specifically, type II neurons display a depolarizing voltage sag under stimulation with negative current steps as well as post-inhibitory rebound (PIR) spiking. The voltage sag reflects the action of the hyperpolarization-activated cation current (I_h) while the PIR is primarily mediated by low threshold Ca-currents (I_T). Our first computational model was designed to reproduce the behavior of type II BNST neurons including the voltage sag, PIR and spike frequency adaptation (Fig. 1A). We refer to this neuron model as the regular firing type. The next model included the inward rectifying K-current ($I_{K_{ir}}$) but no T- or h-currents. A subclass of BNST neurons is known to express strong K_{ir} -currents and are referred to as type III neurons (Hazra *et al.*, 2011) (Szücs *et al.*, 2012). Such neurons display hyperpolarized resting membrane potential (below -70 mV), low input resistance and a characteristic slow onset of firing in response to suprathreshold current injection. Our second model phenotype was designed to reproduce physiological properties of type III BNST neurons and we refer to this model as the delayed firing type (Fig 1F). The third model included a low threshold and slowly inactivating K-current ($I_{K_{slow}}$) that promotes the generation of rhythmic bursting in pacemaker neurons such as GABAergic medial septal neurons (Wang, 2002). This model fired only single spikes at moderate current levels near the rheobase and exhibited bursty rather than regular firing in response to stronger depolarizing current injections. We refer to this model as the stuttering type highlighting the irregular nature of its firing output (Fig. 1K).

Each of the three models included one or two voltage-gated conductances that played a key role in shaping their voltage responses (I_h and I_T for the regular type; $I_{K_{ir}}$ for the delayed firing type; K_{slow} for the stuttering type), but they also shared conductances that were present in 2 or 3 of them. We aimed to explore how up- or downregulation of specific voltage-gated conductances affected the static and dynamic excitability of these biophysically different model neurons.

Static versus dynamic excitability

Our prior electrophysiological study on BNST neurons and simulations with a single-compartmental computational model demonstrated differential regulation of intrinsic excitability under the action of static vs. dynamic inputs (Szücs & Huerta, 2015). In our first set of simulations here we aimed to further characterize these effects in biophysically more realistic models. We performed virtual experiments with two type of stimulus protocols to elicit firing responses of the 3 model cell types. As a conventional approach, intrinsic excitability of neurons is analyzed in experiments with intracellular injection of current pulses of incrementing levels (static stimulation). This was the first type of stimulation we

used in our model simulations. Here, 500 ms steps of somatic current injections were applied and the input-output relationship of neurons was surveyed at 2 pA resolution. Current levels in such model experiments ranged from zero to 2-times of the rheobase of the selected phenotype.

For the second type of stimulation we used barrages of excitatory and inhibitory synaptic conductances, which can be considered as a more realistic approximation to what biological neurons experience in natural conditions. Under such input the neurons fire irregularly and the subthreshold fluctuations of membrane potential reach over 20 mV peak-to-peak (Fig. 2A, D, G). Here, one AMPA- and one GABA_A-type synaptic inputs were simulated both targeting the dendritic compartment of the models (mean rate 50 Hz, Poissonian-distributed). The sweep duration of the synaptic waveform was 8 s and the strength of the excitatory and inhibitory synaptic conductance was gradually strengthened in the successive sweeps. The increment for the AMPA- and GABA-type connections was 0.1 and 0.2 nS, respectively (1:2 ratio). In effect, this protocol simulated a gradual and balanced upscaling of all synaptic connections to the neuron. As demonstrated in Fig. 2B, the number of spikes emitted by the model neuron was steadily increasing in such simulations and an I-O curve analogous to the one in the DC experiment (Fig. 1D) was obtained. This protocol allowed us to survey the dynamic excitability (Szűcs & Huerta, 2015) of the 3 model phenotypes and to characterize the influence of specific voltage-gated currents in regulating their firing responses. Similar to the simulations with current steps we incremented the strength of the synaptic inputs to a level that was twice of the threshold synaptic conductance. Cumulative spike numbers were then obtained by counting the total number of spikes emitted in the stimulus intensity range from threshold to twice the threshold.

Figure 3 demonstrates the static and dynamic excitability profiles of the three types of neurons for 5 selected voltage-dependent membrane currents. Here, normalized cumulative spike numbers are plotted against the maximal conductance of the selected ionic current. Cumulative spike numbers from the generic models served as reference values for these plots (intersections). The maximal conductances were gradually adjusted from 0 nS to +100% of the reference values. The analysis of 5 voltage-gated currents from the 3 neuronal phenotypes largely confirmed our prior results on the less detailed computational model. As an example, the inward rectifying K-current, present in the regular firing and delayed firing models, had a far stronger impact on the dynamic excitability than on the static excitability. Blocking $I_{K_{ir}}$ in the delayed firing type model increased the dynamic and static excitability by 66 and 21 %, respectively (Fig. 3B; K_{ir}). Doubling the maximal conductance of the K_{ir} -current resulted in 50 vs. 27 % decrease of the same parameters. Similar contrasting effects of $I_{K_{ir}}$ were observed in the stuttering type model. As a second example, up- or downregulation of the T-type Ca-current in the first model induces even more contrasting effects in the excitability under static versus dynamic inputs (Fig 3A; Ca_T). Here, up- or downregulation of the T-current exerts a weak effect in the static excitability while regulating the synaptic integration in a far more robust manner (7 vs. 45% change on upregulation). As we have shown in our prior study, some of the voltage-gated conductances behave in the opposite way, they exert a stronger regulatory effect on the current step responses than on the firing under synaptic input. As an example, upregulation of the M-current has a slightly stronger effect on static excitability than the dynamic excitability (Fig.

3A and B; M). This is in agreement with the results of the earlier study (Szücs & Huerta, 2015). The persistent Na-current is included only in the stuttering type model and its impact on firing appears to be stronger under synaptic inputs than under current step stimulation (Fig 3C; I_{NaP}). This is in contrast with the results from the generic BNST type neuron model of our previous study. Another interesting finding is that the interplay between the high-threshold Ca-current (I_{CaL}) and the Ca-dependent K-current produces excitability profiles that are clearly ‘nonlinear’. The degree of changes in static or dynamic excitability by L-current regulation also depends on the cell type. Upregulation of the L-current in the regular firing phenotype results in a steep increase of the dynamic excitability without marked changes in the static responses (Fig. 3A; I_{CaL}). Downregulation of the same current in the delayed firing model attenuates the current step responses (Fig. 3B; I_{CaL}) more effectively than the firing under synaptic inputs.

In conclusion, our simulations with the three biophysically different neuron models show that the regulatory impact of specific voltage-gated currents markedly depends on the type of stimulation (static vs. synaptic) and in a less degree, on the neuronal phenotype. While the general effect of up- or downregulation of a voltage-activated current is quite consistent across neuron types (e.g. membrane depolarization and increased spiking), the findings on static responses rarely provide an accurate estimation for the degree of regulation of synaptic responses.

Frequency dependent regulation of intrinsic excitability

One important difference between current step stimulation and simulated synaptic bombardment is the extent of voltage regime that is visited by the neuron. In the latter case the membrane potential between spikes can reach more negative levels than under constant current inputs. A number of voltage-dependent membrane currents are sensitive to such brief periods of hyperpolarization and their activation or de-inactivation is facilitated by transient inhibition (e.g. I_{CaT} and I_{KIr}). In such conditions, their contribution to shaping the firing output becomes more significant than when the voltage-gated currents are kept inactivated/deactivated due to prolonged depolarization. As we have shown, inspection of the voltage dependence of activation and inactivation of the currents helps explaining their differential regulatory effects under static versus dynamic inputs (Szücs & Huerta, 2015). Additionally, the kinetic properties of activation and inactivation vary greatly across different types of voltage-gated currents. Hence, our next set of simulations addressed the question of how specific voltage-gated currents regulate the excitability when frequency-modulated fluctuating synaptic inputs were applied (Fig. 4). Here, we generated a library of simulated presynaptic spike trains that were presented to the three types of model neurons via AMPA- and GABA-type connections. While the temporal structure of the GABA-input was fixed for all simulations (Poissonian-distributed interspike intervals, Fig. 4B), the excitatory input waveforms were frequency-modulated (Fig. 4A). A total of 51 excitatory presynaptic voltage waveforms (spike trains) were created for the AMPA-connection using a mean firing rate of 180 Hz. A sinusoidal modulation of 150 Hz amplitude was imposed on the mean. Hence, the combined action of the oscillatory AMPA-input and the Poissonian inhibitory background was effectively driving the neurons' membrane potential in a wide regime and in a quasi-periodic manner. The frequency of the oscillatory envelope varied between the low

0.39 Hz (delta range) and 45.3 Hz (medium gamma) across waveforms. It is notable that the net level of excitation and inhibition was virtually the same for each oscillatory pattern, only the temporal structure of the synaptic input was different. The model neurons received spectrally broad-band stimulation through the AMPA- and GABA-connections, but the periodic fluctuations were still clearly discernible in the voltage responses up to gamma frequencies (Fig. 4K-M). To obtain dynamic I-O curves of the neurons excitatory and inhibitory synaptic conductances were gradually incremented up to twice the threshold conductance individually determined for each input pattern. Relative excitability values were calculated for two settings: either removing the selected voltage-gated current or doubling its original maximal conductance. Figure 5 shows the frequency-dependence of relative excitability from such simulations. In each model neuron the effects of five selected voltage-gated currents are analyzed. Filled symbols indicate the relative excitability values from the upregulated models (maximal conductance doubled). Open symbols indicate relative excitability obtained under the removal of the selected voltage-gated current ($g_i=0$ nS). Starting with the low-threshold Ca_T -current in the regular firing model we observe a clear peak in the excitability profile around 6.6 Hz when the Ca_T -conductance is doubled (Fig 5A; Ca_T). While cumulative spike numbers of this neuron model increase by $\sim 30\%$ in the low end of the frequency range (delta-frequencies), we find a $\sim 60\%$ increase at the peak of the excitability curve between 5 and 10 Hz. Blocking the same current results in a marked dip of excitability that mirrors nicely the profile obtained under Ca_T -upregulation. Manipulation of the fast-activating, D-type K-current in the same phenotype yields similar peaked excitability profiles. Blocking the D-current in the regular firing type neuron increases its excitability mostly at theta frequencies (3-10 Hz). At the same time, the up- and downregulation of the M-type K-current yields excitability profiles with dips rather than peaks. The local minimum of relative excitability is observed near 12 Hz frequency of the oscillatory synaptic input after blocking the M-current in the regular firing phenotype. While the upregulation of the L-type Ca-current exerts minor change of firing responses below 2 Hz and above 10 Hz frequencies, we find a clear peak in the theta range. Finally, the Ca-dependent K-current also yields peaked excitability profiles although in a less pronounced manner.

Performing the same analysis using the other two types of model neurons we find similar frequency-dependent excitability profiles. The M-current is included in all three phenotypes and the manipulation of this current produces consistent changes of excitability. We note that upregulation of this current produces excitability profiles with a local maximum around 10 Hz (M-resonance). The location of the dip, i.e. the minimum of excitability vs. frequency function depends on the type of neuron used for the simulations. The inward rectifying K-current changes the excitability most efficiently at theta frequencies in the delayed firing phenotype (Fig. 5B; K_{ir}) while the effect of the D-current is similar to the one we observed for the regular firing model. As a general observation, regulation of the firing responses by voltage-gated currents appears to be less frequency-dependent in the stuttering type model neuron than in the other two types. Here we find less pronounced peaks of excitability profiles when manipulating the maximal conductances of the five selected currents (Fig. 5C).

The peaks and dips of relative excitability curves represent a kind of resonant behavior of voltage-gated currents in regulating the neuronal firing under oscillatory synaptic inputs. In fact, normalized cumulative spike numbers at frequencies of peak locations can be as much as twice higher than outside of that range. Four of the analyzed voltage-gated currents exhibit the strongest effect at theta frequencies while the M-type K-current does essentially the opposite. We find the weakest impact of the M-type K-current near theta frequencies and it becomes stronger when either decreasing or increasing the frequency of the synaptic oscillation.

Analysis of membrane resonance using sinusoidal current input

A number of voltage-activated membrane currents have been shown to promote subthreshold membrane resonance in electrophysiological and computational studies (Hutcheon *et al.*, 1996a). As a common technique to characterize the degree of such resonance, experimenters measure the membrane impedance of neurons under the injection of frequency-modulated sinusoidal current waveforms (Fig. 2C, F, I) (Hu *et al.*, 2002; Heys *et al.*, 2010; Xue *et al.*, 2012). Impedance curves with clear peaks are interpreted as the manifestation of subthreshold membrane resonance. Our next set of simulations addressed the question of how impedance profiles calculated from model experiments can explain the frequency dependence of excitability as observed under the presentation of oscillatory synaptic inputs. To this end, first we used a zap current waveform of 65.8 s duration and its frequency increasing from 0 to 30 Hz in a linear fashion. The amplitude of this waveform was determined separately for each model implementation, i.e. set to the level just 0.1 pA below the firing threshold of the selected model (2 maximal conductances for 5 currents in 3 phenotypes). Impedance curves were obtained from fast Fourier transforms of the input current and the voltage response as a standard method. Our expectation was that changes in the impedance curves of the neurons due to the up- or downregulation of their selected voltage-gated currents will reflect the frequency dependent excitability profiles under synaptic stimulation. Figure 6 shows the summary of results from such calculations. Here, the first, regular firing model exhibits clear subthreshold resonance manifesting as a strong peak in the impedance in the theta range. This is most pronounced when the T-type Ca-current is upregulated (Fig. 6A; Ca_T). Blocking the same current reduces the membrane impedance and the difference between the two curves is the greatest between 1 and 10 Hz. Consequently, we expect a strong increase of excitability due to the upregulation of the T-current in this frequency range, and indeed, this is what we found in our simulations above (Fig. 5A; Ca_T). At the same time, the membrane impedance of this model is not sensitive to the up- or downregulation of the T-current above 10 Hz, yet the relative excitability curves under synaptic inputs appear to be quite divergent for the two settings. We find similar discrepancies for the other voltage-gated currents. Only a minor decrease in impedance is observed under the upregulation of the D-current (Fig. 6A; D), but the bell-shaped excitability profiles are similar to the ones we found for the T-current (Fig. 5A; D). The membrane impedance is clearly reduced by the upregulation of the M-current below theta frequencies and this is in agreement with the decrease of relative excitability under synaptic inputs. At the same time, the impedance curves tightly overlap at the higher frequencies while the excitability profiles display increasing divergence again. Hence, either doubling or blocking the M-current exerts minor changes in the observed membrane impedance above

10 Hz, but the relative excitability of the same model changes by -50 vs. +100 %, respectively (Fig. 5A; M). We also find that membrane impedance is almost completely insensitive to the manipulation of the L-type Ca-current or the K(Ca)-current (Fig. 6A and B; Ca_L and K(Ca)) - another contrast with the observations on the excitability under oscillatory synaptic inputs. Turning to the other two neuronal phenotypes, we find a similar pattern. Interestingly, the manipulation of the K_{ir} -current in the delayed firing neuron results in nicely diverging impedance curves and they remain separate in the entire frequency range. The greatest difference between these curves is found at theta frequencies and this would correctly predict the changes in excitability by up- or downregulating the K_{ir} -current. The other four voltage-gated currents have a considerably weaker impact on the impedance curves all being monotonously decreasing functions of the frequency. The third model phenotype (stuttering cell) exhibits the greatest separation of impedance profiles when manipulating the K_{slow} -current. As we showed above using oscillatory synaptic inputs, this phenotype lacks the kind of resonant behavior that was revealed for several currents in the previous two model phenotypes. Consistent with the other phenotypes, impedance curves of the stuttering type neuron tend to decrease at higher frequencies and separation between the two curves is also reduced.

These findings demonstrate that conventional analysis of subthreshold membrane resonance does not correctly predict the frequency-dependent changes of relative excitability under the manipulation of most intrinsic membrane currents in our model phenotypes. With the exception of the K_{ir} -current, the impedance profiles above theta frequencies are virtually insensitive to the changes in the magnitude of the voltage-gated currents. Still, excitability profiles exhibit clear separation under such manipulations.

Intrinsic excitability studied under the manipulation of passive membrane properties

In addition to the firing properties, voltage-gated currents influence physiological parameters of neurons that are commonly measured at rest, such as their passive membrane properties. In particular, currents that activate at potentials negative to spike threshold can alter the neurons' resting membrane potential, input resistance and membrane time constant. As an example, a strong inward rectifying K-conductance, being partially activated at rest, hyperpolarizes the neuron and lowers its membrane resistance. One could argue that some of the physiological effects observed under the upregulation of voltage-dependent membrane currents might be reproduced by changing the passive membrane properties of the computational model. Hence, it is possible that the frequency-dependent regulation of intrinsic excitability by voltage-dependent currents arises partially due to their impact on membrane resistance and time constant. Additionally, the average level of membrane potential during the presentation of the synaptic input might have an impact on the activation and inactivation properties of the membrane currents. In our next set of simulations, we examined how manipulations of the passive membrane properties of the three neuronal phenotypes influenced their firing under stimulation with oscillatory synaptic inputs. We calculated normalized cumulative spike numbers from synaptic responses under the manipulation of either the leakage conductance, the membrane capacitance, the leakage reversal potential or by applying a constant DC current to shift the average membrane potential. These model simulations essentially produced flat excitability profiles (Fig. 7)

rather than peaks or U-shaped functions such as observed under the up- or downregulation of specific voltage gated currents. With the exception of g_{leak} , normalized cumulative spike number plots exhibit no frequency-dependence suggesting that the membrane capacitance and leakage reversal potential parameters have minor role in shaping the resonant properties of the cell. Lowering the leakage conductance increases the intrinsic excitability in a non-uniform manner. Here we observe slight excess at higher frequencies, i.e. firing responses under beta- and gamma-frequency oscillatory inputs are more sensitive to changes in the membrane resistance, especially in the regular and delayed firing models (Fig. 7A and B).

Insertion of synthetic K_{ir} -current in dynamic clamp

Computational modeling allows us to perform virtual experiments based on a variety of stimulus protocols and to manipulate the model neurons intrinsic biophysical properties. Here, one can conveniently obtain the model neurons' high-resolution I-O profiles that would require hours of stimulation and recording in real-world conditions. Nevertheless, testing some of the model predictions on biological neurons can be still attained. One can take advantage of the dynamic clamp technique to insert a synthetic voltage-dependent membrane current into biological neurons (Vervaeke *et al.*, 2006; Szűcs & Huerta, 2015) while concurrently exposing them to simulated synaptic bombardment. Indeed, our earlier data on extended amygdala neurons proved the value and utility of this approach (Szűcs *et al.*, 2010). Hence, we designed dynamic clamp protocols based on the approach shown in the model simulations. For these experiments we used cultured rat hippocampal neurons that exhibit many characteristics of the regular firing phenotype. They were subjected to simulated synaptic bombardment in whole-cell conditions. The neurons were previously isolated from their endogenous synaptic inputs using a combination of blockers of glutamatergic and GABAergic transmission. These cells ($n=11$) exhibited no significant degree of inward rectification under negative current steps and their firing under depolarizing current steps was not delayed as in neurons expressing a strong endogenous K_{ir} -current (Fig. 8M). Insertion of the dynamic clamp-computed artificial K_{ir} -current ($g_{K_{\text{ir}}}=20$ nS) distinctly changed the physiological character of these neurons as demonstrated in Fig. 8N. Their voltage responses became similar to those of the delayed firing type model neurons (Fig. 1F), they exhibited decreased membrane resistance and prominent latency to the first spike under suprathreshold depolarizing current steps. Here, constant current steps as external stimulation and the synthetic K_{ir} -current were simultaneously computed and delivered by the dynamic clamp system through the recording electrode. We selected three frequencies (referred to as delta-, theta and beta-frequencies) for the oscillatory synaptic input which allowed us to obtain a low-resolution sampling of the excitability profiles of the neurons (3 instead of 51 points). By limiting the duration of such dynamic clamp experiments we aimed to record the firing responses within the temporal window of stable whole-cell conditions (typically 10-12 min). Voltage output of the hippocampal neurons receiving the three types of oscillatory synaptic inputs are shown in Fig. 8A. Here, the strength of the excitatory and inhibitory connections was gradually increased resulting in membrane potential fluctuations with increasing amplitude. The duration of each synaptic input was 4 s, half of that in the model simulations, while the increment of g_{AMPA} was 2 nS yielding the I-O curves shown in Fig. 8B-D. As in the model simulations, cumulative spike numbers were obtained between the threshold AMPA-

conductance and twice of that value. Next, we inserted the synthetic K_{ir} -current and performed the same protocol (Fig. 8E, F-H). The threshold AMPA-conductance of firing clearly shifted to the right for all 3 frequencies of synaptic oscillation after the insertion of the K_{ir} -current. Besides, the amount of threshold shift was the greatest for the theta-frequency type synaptic input. Fig. 8I summarizes these threshold shift data from all 11 neurons and Fig. 8J displays the results of the statistical comparison. Paired t-tests for means showed marked difference between the effects at the theta- vs. delta-type inputs (0.99 ± 0.21 , 2.13 ± 0.28 and 1.99 ± 0.17 nS; delta-, theta- and beta-input, respectively). In addition to the shift in threshold, the impact of K_{ir} -insertion on cumulative spike numbers was the greatest at the middle frequency (Fig. 8K, L; 0.51 ± 0.06 , 0.24 ± 0.06 and 0.30 ± 0.05) demonstrating that this voltage-dependent current attenuates firing most effectively at theta-frequencies of the synaptic oscillation. These observations well agree with the data from the model simulations on the delayed firing phenotype.

Discussion

In this study we analyzed the firing responses of three types of model neurons using static current stimuli and under simulated, frequency-modulated synaptic bombardment. Neuronal intrinsic excitability was regulated by specific voltage-dependent currents in a frequency-dependent manner mostly centering at theta frequencies of the synaptic input. Electrophysiological experiments using simulated synaptic bombardment of cultured hippocampal neurons largely confirmed model predictions for a selected voltage-gated current and demonstrated the generality of this phenomenon.

Means of quantifying intrinsic excitability

While intrinsic excitability of neurons and its regulation by neuromodulators, homeostatic or neuroadaptive mechanisms has long been studied (Desai *et al.*, 1999; Fan *et al.*, 2005; Turrigiano, 2011), it is rarely addressed how changes in the excitability of neurons translates to their firing under various types of synaptic inputs. Commonly, researchers have been using incrementing levels of intracellular current steps to measure the firing responses of neurons. Changes in the rheobase or the input-output gain are interpreted as signs of alterations of the neurons' intrinsic excitability (Chance *et al.*, 2002). As an alternative to static current steps, one could use more realistic means of stimulation, such as incrementing levels of an excitatory synaptic input (John & Manchanda, 2011). However, accurately controlling the strength and temporal structure of synaptic excitation and deriving input-output functions from such experiments is challenging. Alternatively, one can utilize dynamic clamp methods (Szűcs & Huerta, 2015) to meet these requirements or perform virtual experiments with model neurons as we did here. One key difference between the two experimental approaches is that the membrane potential fluctuates in a wider range under the action of synaptic inputs than when using constant levels of intracellular current. To characterize the intrinsic excitability of neurons we preferred to use the normalized cumulative spike number as a relative measure. This way we were able to detect even slight changes in the firing responses in a relatively wide range of stimulus intensity and to compare the impact of various voltage-gated currents under different types of inputs (current steps vs. synaptic input). Hence, the impact of up- or downregulation of an intrinsic

membrane current could be accurately assessed and compared for different functional regimes of the neuron.

Differential regulation of excitability

Recently, we have demonstrated differential regulation of intrinsic excitability by voltage-dependent membrane currents both in biological neurons of the rodent extended amygdala and in a simple computational model (Szűcs & Huerta, 2015). By inserting an artificial, computer-simulated inward rectifying K-current into type II BNST neurons via dynamic clamp we have shown that the current step responses were less sensitive to such manipulation than the neurons' firing under simulated synaptic bombardment. In the present study we further characterized the differential effects of voltage-gated membrane currents in three, biophysically different model neurons. Again, we found contrasting effects of voltage-gated currents in regulating the firing responses under static vs. dynamic inputs. In agreement with the results of the previous study we found that the K_{ir} -current exhibited the most diverging excitability profiles, i.e. this current regulated synaptic integration far more effectively than the firing responses under current steps. Interestingly, the impact of the high-threshold Ca-current (L-current) was quite variable in various conductance regimes resulting in peculiar “nonlinear” excitability profiles. We recognize that the L-current plays a fundamental role in gating the K(Ca)-current and the interplay of these two likely contributes to the complex regulation of firing. We also find that the divergent behavior of excitability profiles is a collective feature of the three neuronal phenotypes. While the model neurons are composed of different types of voltage-gated currents and they exhibit markedly different physiological properties (voltage sag, PIR, etc.), their excitability is regulated by specific currents in a fairly consistent manner. Whenever a current is shared by two or three model phenotypes (e.g. the K_{ir} -, M- and D-currents), we find similar degrees of divergence in the static vs. dynamic excitability profiles of those. Nevertheless, cell type specific differences are also revealed, like for the L-type Ca-current in the regular and delayed firing neurons. Here, upregulation of the current strongly increases the dynamic excitability of the regular firing model while downregulation of the same current has a much stronger effect in the static excitability of the delayed firing type. These findings indicate that plastic or neuroadaptive changes in the strength of specific voltage-dependent currents can impact the excitability of neurons in a differential manner for physiologically different types of neurons.

As one of the voltage-gated currents investigated extensively by neurophysiologists, the hyperpolarization-activated cation current, I_h has been shown to regulate intrinsic excitability in such manner. The h-current commonly decreases intrinsic excitability by its shunting effect and by lowering the membrane time constant (Poolos *et al.*, 2002; Fan *et al.*, 2005). Nonetheless, it has been shown that it can also increase both static excitability and synaptic responses by slightly depolarizing the somatic and dendritic membrane (Dyhrfeld-Johnsen *et al.*, 2008). Our prior and present modeling results with the I_h support the second possibility (Szűcs & Huerta, 2015). These contrasting effects can manifest as differential regulation of firing responses under different type of stimulation or in different cell types.

Regulation of resonant properties by voltage-gated currents

The simulations with frequency-modulated synaptic inputs revealed a wide repertoire of resonant behavior that were not readily anticipated from the analysis of subthreshold voltage responses under ZAP stimulation. As a particularly interesting case, the delayed firing type neuron is expected to behave as a low-pass filter considering its impedance profile. With the exception of $I_{K_{ir}}$, up- or downregulation of its voltage-dependent currents have minor impact on the membrane impedance and one would not expect notable changes of intrinsic excitability in the theta range for this phenotype. Nevertheless, this is what we find when manipulating the M- and D-currents and to a less degree, the L-type Ca-current. Also, membrane impedance is nearly unaffected by the manipulation of the D-current in the regular firing neuron while the dynamic excitability profiles indicate robust changes in the theta range again. Altogether, we manipulate voltage-dependent currents and survey excitability profiles in 15 conditions (5 currents for each cell type) and observe significant changes of membrane impedance only in 6 cases. However, robust, frequency-dependent regulation of excitability is observed in 11 scenarios clearly showing a discrepancy between the subthreshold and suprathreshold data.

A variety of potassium currents have been associated with neuronal resonance properties. While Kv7 channels, mediating the M-current, have been shown to promote theta-frequency membrane resonance in pyramidal neurons (Peters *et al.*, 2005; Hu *et al.*, 2009) and stellate cells (Heys *et al.*, 2010), Kv1 channels, mediating the D-current are reportedly associated with gamma-frequency resonance (Hsiao *et al.*, 2009; Sciamanna & Wilson, 2011). In our simulations the two currents exhibit contrasting effects on excitability. Upregulation of the D-current exerts the strongest inhibition of firing output at low theta frequencies. The M-current does just about the opposite: it exerts its strongest inhibition outside of the theta range, actually promoting firing at theta frequencies (M-resonance) (Hönigsperger *et al.*, 2015). Considering the activation properties, the K_{ir} - and M-currents are more comparable - or symmetrical - because they are both non-inactivating type currents and have steady-state activation functions with opposite inclination. As we found, their excitability profiles obtained under oscillatory synaptic inputs also reflect this symmetry.

Surprisingly, the location and magnitude of peaks of excitability profiles do not match the shape of impedance curves for several of the intrinsic membrane currents studied in our simulations. What can cause this discrepancy between subthreshold membrane resonance and the frequency-dependent regulation of firing responses? The experimental procedures aiming subthreshold resonance are based on the analysis of voltage output when the current stimulation is kept below the firing threshold or action potentials are prevented by Na-channel blocking. Under the ZAP protocol, the neuron is exposed to intracellular current injection of a frequency-modulated sinusoidal waveform, but it can be considered as narrow-band stimulation in any given timepoint. Also, resonance measurements assess the subthreshold (small-signal) responses of neurons largely ignoring their strongly nonlinear properties that become dominant near spike threshold. At the same time, spiking resonance is studied in conditions when the neuron is firing intensely under broad-band, frequency-modulated synaptic inputs. Another difference between the two scenarios is that the peak-to-peak membrane potential fluctuations under ZAP stimulation are typically higher than under

synaptic bombardment (Fig. 2 vs. Fig. 4). The membrane potential under sinusoidal current injection can visit levels below the reversal potential of inhibitory synaptic inputs ($E_{\text{GABA}} = -72$ mV in our simulations). All these differences can contribute to the contrasting observations on resonant properties under the analysis of subthreshold membrane impedance and the dynamic excitability profiles we presented above.

The consistent results on three biophysically different model phenotypes and the dynamic clamp data suggest that resonant-type regulation of intrinsic excitability by voltage-gated currents might be a general phenomenon manifesting in a multitude of biological neuron types. However, one can ask how robust the excitability profiles are against changes in the activation properties or kinetics of the intrinsic currents or synaptic inputs. While exploring the effects of manipulations of all biophysical parameters is out of the scope of the current study, we performed additional simulations addressing the impact of the potentially most important kinetic parameters. As an example, the excitability profiles of the Ca_T -, M- and K_{ir} -currents (as in Fig. 3) remain virtually unaffected by reducing the time constants of the AMPA- and GABA-connections by 60 % or by increasing those by 100 % (Fig. S1). At the same time, when the activation and inactivation time constants of the above 3 intrinsic currents are manipulated, we observe changes of the locations of the resonant frequencies. Faster Ca_T -, M- and K_{ir} -currents promote spiking resonance at higher frequencies of the oscillatory synaptic input (beta- rather than theta-frequencies; Fig. S2). Additionally, changing the voltage-dependence (steady-state activation functions) of the currents without manipulating their maximal conductances exerts moderate effects on the relative excitability (Fig. S3). These observations suggest that the frequency-dependent regulation of intrinsic excitability is closely related to the kinetic, rather than voltage-dependent properties of the intrinsic membrane currents.

Functional consequences

Our findings demonstrate that even when specific voltage-gated currents do not appear to contribute significantly to subthreshold resonance, they can still regulate the postsynaptic firing rate in a clearly frequency-selective manner. For example, T-type Ca-channels have been recently shown to amplify resonance in inferior olive neurons primarily mediated by HCN channels (Matsumoto-Makidono *et al.*, 2016). Also, inward rectifying K-channels, while less often studied in the context of rhythm generation, have been shown to tune the frequency-response of striatal medium spiny neurons (Zhao *et al.*, 2016). The many different ways to generate resonance in neurons have been discussed in the context of the activation properties and kinetics of voltage-gated membrane currents (Hutcheon & Yarom, 2000). Accordingly, most voltage-dependent currents can be classified as being resonant or amplifying types allowing the manifestation of some degree of resonance in virtually all neurons. Yet, the frequency-selective regulation of intrinsic excitability as we showed here is likely a higher-order phenomenon that is not a direct consequence of subthreshold resonance. The resonant type regulation of firing rate by voltage-gated currents can be a very effective way to tune the overall activity and synchronization of neuron populations under frequency-modulated synaptic inputs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Analysis of the physiological parameters of the three model cell phenotypes. The voltage traces on the left (A, F, K) show the responses of the model neurons under the stimulation with current steps of incrementing levels. The regular firing model exhibits distinctive postinhibitory rebound due to the activation of the T-type Ca-currents (A). Delayed onset of firing in the second phenotype is produced by the deactivation of the inward rectifying K-current under depolarizing current steps (F). The 3rd type of model fires action potentials in a bursty, ‘stuttering’ pattern (K). Panels B, G and L show the membrane resistance as a function of the input current for each phenotype. Resistance values are calculated either from maximal voltage deflection data (black arrows; $R_{\max}$) or voltage samples recorded at the end of the current steps (gray arrows; R_{end}). Input-output relationship plots of the corresponding models are shown in D, I, N. Voltage sag (black) and afterdepolarization (gray) are plotted against the input current in C, H and M. The hyperpolarization-activated cation current I_h mediates the prominent voltage sag seen in the regular firing (C) and stuttering type (M) models. Slight voltage sag in the delayed firing model develops at current levels below -100 pA as a result of the activation of the inward rectifying K-current (H) but no afterdepolarization is observed in this cell type. Latency to the first spike as a function of the input current is shown in E, J and O.

Figure 2.

Analysis of dynamic excitability and subthreshold resonance in the three model neurons. Voltage responses under simulated synaptic bombardment are shown in A, D and G at $g_{AMPA} = 12$ nS and $g_{GABA} = 24$ nS levels. We observe gradually increasing number of spikes under stimulation with incrementing levels of synaptic conductances (B, E, H; white symbols: generic models). The number of emitted spikes is strongly reduced after doubling the maximal conductance of the intrinsic M-current in all three neurons (black symbols). M-current upregulation has the strongest effect in the regular firing model (B). Rightmost panels (C, F, I) are voltage responses of the corresponding models under the injection of frequency-modulated sinusoidal current waveforms (ZAP). The regular firing model exhibits the strongest subthreshold resonance. The stuttering type model exhibits two peaks in the voltage waveform, spiking occurs at the beginning of the ZAP waveform (asterisk).

Figure 3.

Voltage-gated membrane conductances regulate the intrinsic excitability of neurons in a differential manner. In each plot normalized cumulative spike numbers are plotted against the maximal conductance of a selected membrane current for two types of stimulation (currents steps: open squares, simulated synaptic bombardment: filled circles). Excitability profiles for static versus dynamic stimulation clearly diverge for several voltage-gated currents (e.g. the Ca_T -, D -, K_{ir} and $K(Ca)$ -currents). The excitability profiles intersect in the middle where the conductance is set to the value of the generic model phenotype (reference cumulative spike numbers).

Figure 4.

Examples of oscillatory synaptic inputs and the voltage responses of the regular firing type neuron model. A and B show 3s sections of the conductance waveforms for rhythmic excitation and tonic inhibition. The AMPA waveform is frequency-modulated at 4.60 Hz in this example. C is the computed net synaptic current while these conductance inputs are coupled to the regular firing type model neuron in D. Maximal synaptic conductance (g_{AMPA} and g_{GABA}) were 8 and 16 nS allowing spiking in this example. Grayed sections of the waveforms are displayed in the zoom plots on the right. Panels E, F and G show subthreshold voltage responses of the regular firing type model at 4 nS AMPA and 8 nS GABA levels for three different modulatory frequencies. Fourier amplitude spectra calculated from the corresponding synaptic current traces and voltage responses are shown in H-J and K-M, respectively.

Figure 5.

The impact of voltage-dependent membrane currents in regulating the firing depends on the frequency of the oscillation embedded in the excitatory synaptic input. Here, relative cumulative spike numbers of 5 voltage-gated currents are shown as functions of the frequency of the oscillatory envelope for three model phenotypes. For each plot normalized cumulative spike numbers were calculated at two levels of the maximal conductance of the selected current: either at twice the level of the original conductance or at zero (filled vs. open symbols). Several of the voltage-gated currents exhibit a characteristic peak in their excitability profiles at theta frequencies (4-7 Hz). Voltage-gated currents in the stuttering type model display relatively flat frequency dependence in their excitability profiles (C). Frequency values appearing in the plots indicate the estimated location of the peaks or dips.

Figure 6.

Analysis of the subthreshold membrane resonance under the manipulation of voltage-gated currents in the three model phenotypes. Maximal conductances of selected membrane currents were up- or downregulated as in Fig. 5 (filled vs. open symbols). The first, regular firing phenotype exhibits stronger membrane resonance (A) than the other two types (B and C). Upregulation of the T-type Ca-current augments the resonant behavior (peak at 4.5 Hz) while the other currents have weaker effects in the regular firing type model. Impedance is increased by the doubling of the T-current and reduced when the M-current is upregulated. The K_{ir}-current in the delayed firing model strongly reduces the membrane impedance in a wide frequency range, while the M-type current is effective only at sub-theta frequencies. The stuttering type model exhibits relatively flat impedance curves (C) and the K_{slow}-current is the most effective in regulating the membrane impedance of this phenotype.

Figure 7.

Manipulation of the passive membrane properties promote uniform changes in intrinsic excitability of the three model phenotypes. The membrane resistance of the models was altered by up- or downregulating their leakage conductance (g_{leak}) by 30 % (filled vs. open teal colored symbols). Here, we find local maxima and minima in the excitability profiles at higher frequencies (20 Hz and above). Increase or decrease of the capacitance of the somatic compartment by 50 % uniformly changes the excitability of the model neurons in the entire frequency range (filled vs. open maroon colored symbols). A similar effect is seen when the reversal potential of the leakage current is shifted up or down by 4 mV (black symbols) or when a DC bias current is applied concurrently with the synaptic stimulation (green symbols; ± 15 pA for the regular and delayed firing types, ± 20 pA for the stuttering type). In the latter case the average level of membrane potential shifts up and down by ~ 3 mV due to the DC current.

Figure 8.

Insertion of an artificial K_{ir} -conductance decreases the firing of cultured hippocampal neurons in a frequency-dependent manner. A shows representative e firing responses of a hippocampal neuron under the action of simulated oscillatory synaptic inputs. Three frequencies of synaptic oscillation were used corresponding to delta-, theta- and beta rhythms. The 3 adjacent traces show responses at 3 selected synaptic conductances (g_{AMPA}), indicated on the left. Panels B, C and D show the input-output functions obtained from such dynamic clamp experiments for the 3 frequencies of oscillation. Traces in E are analogous to those in A, but here an artificial K_{ir} -conductance ($g_{K_{ir}}=20$ nS) was concurrently inserted in the neuron. The corresponding I-O functions reveal a significant decrease of firing due to the action of the computer-generated K_{ir} -current. Data from 11 such experiments are summarized in I-L. The synaptic conductance threshold of firing was increased for all three types of stimulation. Panel I shows the degree of shift of threshold for the delta-, theta and beta-inputs. J shows the average values from data in J and results of paired t-tests. K is the relative excitability calculated analogously to that in Fig. 5. The strongest decrease of relative cumulative spike numbers is observed at the theta-type input. Panels M and N demonstrate the effect of K_{ir} -insertion on the current step responses of another hippocampal neuron. The spiking is clearly delayed and the membrane resistance is decreased due to the action of the artificial K_{ir} -current (compare to Fig. 1F).

Table 1

Passive membrane parameters of the three neuron models. g_{sx} is the electrical coupling conductance between the soma and axon compartments, g_{sd} indicates the coupling between the soma and dendrite.

	Soma		Axon		Dendrite				
	g_{leak} nS	C_m pF	g_{leak} nS	C_m pF	g_{leak} nS	C_m pF	g_{sx} nS	g_{sd} nS	E_{leak} mV
Regular	2.0	60	1.5	20	1.0	10	18	16	-66.0
Delayed	2.0	60	1.5	20	1.0	10	18	16	-64.0
Stuttering	2.5	40	1.5	15	1.0	10	20	16	-62.0

Table 2

Parameters of the voltage-dependent currents for the three neuronal phenotypes (R: regular firing, D: delayed firing, S: stuttering). Som, Axn and Den indicate the percentage of conductance allocated for the somatic, axonic and dendritic compartments.

Current	Type	g nS	E mV	Som %	Axn %	Den %	p	V _{m,1/2} mV	V _{m,d} mV	V _{h,1/2} mV	V _{h,d} mV	τ _{m,max} ms	τ _{m,min} ms	V _{hm,1/2} mV	τ _{h,max} ms	τ _{h,min} ms	V _{hm,1/2} mV	V _{h,d} mV
Na	R	8000	55	25	75	0	3	-27	14	-54	-14	0.9	0.1	-68	8	8	5	30
	D	9000																
	S	9000																
Na _p	R	-	50	33	0	67	1	-26	12			3.0	0.2	-70	50			
	D	1.0																
	S	1.0																
H	R	3.0	-40	33	0	67	1	-73	-16			200	15.0	-62	30			
	D	-																
	S	3.0																
K _d	R	400	-72	25	75	0	4	-22	15			10	0.7	-70	30			
	D	400																
	S	250																
M	R	8.0	-75	50	50	0	1	-28	17			80	20.0	-80	100	100	-75	50
	D	8.0																
	S	5.0																
D	R	200	-80	50	50	0	3	-31	20	-73	-15	2.5	0.2	-50	30	100		50
	D	150																
	S	-																
K _r	R	-	-85	67	0	33	1	-80	-15			50	3.0	-45	40			
	D	8.0																
	S	2.0																
K _{slow}	R	-	-75	50	50	0	1	-26	12	-59	-13	6	6	-60	30	500	50	-30
	D	-																
	S	300																
Ca _T	R	25	90	100	0	0	2	-49	14	-83	-13	5.0	0.8	-68	30	50	-75	25

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Current	Type	g	E	Som	Axn	Den	p	$V_{m,1/2}$	$V_{m,d}$	$V_{h,1/2}$	$\tau_{m,max}$	$\tau_{m,min}$	$V_{h,1/2}$	$\tau_{h,max}$	$\tau_{h,min}$	$V_{th,1/2}$	$V_{th,d}$		
		nS	mV	%	%	%		mV	mV	mV	ms	ms	mV	ms	ms	mV	mV		
Ca _L	D	-	90	50	0	50	2	-23	13	-53	-13	10	1.0	-54	27	50	5	-65	27
		-																	
	S	-																	
		-																	