Differential effects of static and dynamic inputs on neuronal excitability

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Szung, A., Huerta, R. Differential effects of static and dynamic inputs on neuronal excitability. J Neurophysiol 113: 232–243, 2015. First published October 1, 2014; doi:10.1152/jn.00226.2014.——The intrinsic excitability of neurons is known to be dynamically regulated by activity-dependent plasticity and homeostatic mechanisms. Such processes are commonly analyzed in the context of input-output functions that describe how neurons fire in response to constant levels of current. However, it is not well understood how changes of excitability as observed under static inputs translate to the function of the same neurons in their natural synaptic environment. Here we performed a computational study and hybrid experiments on rat bed nucleus of stria terminalis neurons to compare the two scenarios. The inward rectifying K+ current (I_{K_\text{ir}}) and the hyperpolarization-activated cation current (I_h) were found to be considerably more effective in regulating the firing under synaptic inputs than under static stimuli. This prediction was experimentally confirmed by dynamic-clamp insertion of a synthetic inwardly rectifying K+ current into the biological neurons. At the same time, tonic currents that activate with depolarization were more effective regulating the firing under static inputs. When two intrinsic currents are concurrently altered such as those under homeostatic regulation, the effects in firing responses under static vs. dynamic inputs can be even more contrasting. Our results show that plastic or homeostatic changes of intrinsic membrane currents can shape the current step responses of neurons and their firing under synaptic inputs in a differential manner.

INTRINSIC EXCITABILITY, as one of the most distinctive properties of neurons, refers to their propensity to generate action potentials in response to depolarizing current or excitatory synaptic inputs. This behavior reflects the concerted action of voltage-dependent ionic channels in the membrane of the neuron. Beyond the classic spike-generating conductances, i.e., the transient Na and the delayed rectifier type K channels, neurons express a variety of additional voltage-gated channels that regulate the rate and structure of their firing. Neuronal output is therefore produced by a complex interplay between the synaptic inputs and the voltage-dependent membrane conductances. An increasing number of studies demonstrate that intrinsic cellular properties of neurons are subject to activity-dependent plastic changes and homeostatic regulation similarly to those of synaptic properties (Hyun et al. 2013; Jung and Hoffman 2009; O’Leary et al. 2010; van Welie et al. 2004). In this respect, learning, memory, and various other forms of activity-dependent plasticity are also strongly linked to intrinsic excitability and the operation of the voltage-gated channels (Desai et al. 1999; Schulz 2006; Turrigiano 2011).

Experimenterers studying neuronal excitability frequently use intracellular current injection as a standard tool to analyze the firing responses of neurons and the regulation of excitability by specific voltage-gated currents. It is less often addressed how alterations of neuronal excitability as observed in current-clamp experiments translate to the firing of the same neurons when they are functioning in their natural synaptic environment (John and Manchanda 2011; Moyer et al. 2007). Findings from our recent experiments on three types of neurons from the bed nucleus of stria terminalis (BNST) suggested that adaptive changes in intrinsic cellular properties of neurons might regulate their firing responses in a differential manner (Szücs et al. 2012). Specifically, comparing groups of neurons from normal animals and those with chronic alcohol dependence we find no significant changes in the parameters characterizing the neurons excitability under static current inputs. However, when the same neurons are subjected to simulated synaptic bombardment in dynamic clamp, we find a significant decrease of firing output in the treated group interpreted as a reduction of their dynamic excitability. This observation suggests that adaptive changes in the intrinsic cellular properties of neurons regulate their firing in a way that depends on the temporal structure of the input they receive.

In the present work we use computational modeling and electrophysiological experiments to investigate how manipulation of the magnitude of five voltage-gated currents alters the neurons’ excitability. The comparison of data from our experiments with static current stimuli and under simulated synaptic bombardment reveals differential effects on excitability and it suggests that homeostatic regulation of cellular properties can tune the input-output (I-O) transformation of neurons in a pattern-sensitive manner.

MATERIALS AND METHODS

Intracellular recording and dynamic clamp in BNST neurons.

Acute brain slices were prepared as previously described (Francesconi et al. 2009; Szücs et al. 2010). The animal protocols were consistent with guidelines issued by the National Institutes of Health and approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute (Protocol No. 07–0068). Coronal rat brain slices (350 μm) were collected from the rostral cerebrum of Wistar rats using a Campden vibrating microtome (Loughborough, England) in oxygenated artificial cerebrospinal fluid (ACSF) consisting of the following (in mM): 130 NaCl, 3.5 KCl, 24 NaHCO3, 1.25 NaH2PO4, 2.2 CaCl2, 10 d-glucose, and 2 MgSO4, pH 7.4. The slices were preincubated in ACSF for 1 h at 32°C and then maintained at room temperature for at least 30 min before being transferred to a submerged recording chamber (32°C). Slices of brain tissue containing the BNST were placed in a superfusion chamber and visualized with a Nikon differential contrast microscope. Current-clamp and dynamic-clamp experiments were performed in whole cell configuration using 7- to 8-MΩ patch pipettes filled with intracellular solution containing the following (in mM): 120 KMeSO4, 10 KCl, 3 MgCl2, 10 HEPES,
Kir returned to a rest state of 5-ms wide spike-shaped voltage transients that departed from and activity of two presynaptic neurons. The voltage waveforms consisted in inhibitory synaptic inputs as described in Szücs et al. (2010). We used by stimulating them with a barrage of simulated excitatory and membrane potential of the cell. The voltage responses of the BNST from bridge mode. Stimulus waveforms were generated using the data acquisition software DASYLab 11.0 (Dasytec, Amherst, NH) in a Windows computer. We used standard rectangular current commands as stimuli for the initial physiological characterization of BNST neurons. Specifically, we delivered 350-μs pulses of current starting from −200 pA and incremented in 10-pA steps from the resting membrane potential of the cell. The voltage responses of the BNST neurons were analyzed using a software we developed in Delphi 2009 (Szücs et al. 2012).

In dynamic clamp we elicited firing activity in the BNST neurons by stimulating them with a barrage of simulated excitatory and inhibitory synaptic inputs as described in Szücs et al. (2010). We used two computer-generated voltage waveforms to simulate the firing activity of two presynaptic neurons. The voltage waveforms consisted of 5-ms wide spike-shaped voltage transients that departed from and returned to a rest state of −60 mV. To induce variable amplitude synaptic currents in the postsynaptic BNST neuron, we introduced amplitude variation of the spike-shaped voltage transients such that their peak value ranged from −30 to 0 mV in a uniform distribution. Firing rate of these waveforms was 30 Hz, and interspike intervals were drawn from a Gaussian distribution with 20% standard deviation. The input from the first, excitatory voltage waveform was used to evoke rapid (AMPA-type) and slow [N-methyl-D-aspartate (NMDA)-type] excitatory conductance transients. We used simulated chemical synaptic connections (see Nowotny et al. 2006 for details) to couple the excitatory inputs to the biological neuron. The synaptic time constant was 10 and 50 ms for the AMPA- and NMDA-type connections, respectively, and the reversal potential was 0 mV for both. The second voltage waveform served as the GABAergic inhibitory input (\(V_{\text{syn}} = -68\) mV, \(\tau_{\text{syn}} = 10\) ms). We used equal conductances for the NMDA and GABA inputs (AMPA and NMDA type) and half of that for the AMPA input (e.g., 5/10/10 nS). The duration of the presynaptic waveforms was 5 s, and they were repeatedly presented every 13 s (frozen noise protocol); hence, the neurons were at rest for 8 s between stimuli. We investigated the effects of a synthetic Kir conductance on the excitability of BNST neurons while exposing them to either current step stimulation or simulated synaptic bombardment. The synthetic conductance was first-order hyperpolarization-activated and noninactivating type as described in Table 1. Our dynamic-clamp system allowed the insertion of the synthetic \(K_r\) conductance during the concurrent stimulation with current steps or simulated synaptic bombardment. The synthetic \(K_r\) and synaptic currents in dynamic clamp were calculated using the same formalism that we used for the computational model below.

**Computational neuron model.** We designed a single compartmental neuron model consisting of seven voltage-dependent membrane conductances with no Ca dynamics. The passive membrane resistance of the generic model was set to 370 MΩ (2.7-nS leakage conductance) while the membrane capacitance was 80 pf. The voltage dependence and kinetic parameters of the currents were fixed across model experiments, but the maximal conductances of five selected currents were varied to study their impact on the firing responses of the model. All currents were calculated as

\[
I_i = g_i m^p h_i (E_i - V_m),
\]

where \(i\) represents the individual current type, \(g_i\) is the maximal conductance of the current, \(m\) is the activation variable, \(p\) is the exponent of the activation term, \(h_i\) is the inactivation variable (always first-order or absent), and \(E_i\) is the reversal potential. Differential equations for the activation \((m)\) and inactivation \((h)\) are the following:

\[
\frac{dm}{dt} = \frac{m_s(V_m) - m}{\tau_m(V_m)}; \quad \frac{dh}{dt} = \frac{h_s(V_m) - h}{\tau_h(V_m)},
\]

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

\[
m_s(V_m) = \frac{1 + m_l(V_m)}{2}; \quad h_s(V_m) = \frac{1 + h_l(V_m)}{2},
\]

where \(m_l\) and \(h_l\) are tanh functions and shown in Table 1. Time constant of the activation and inactivation are bell-shaped functions of the membrane potential \((V_m)\):

\[
\tau_m(V_m) = (\tau_{m,\text{max}} - \tau_{m,\text{min}}) \left[ 1 - m_l^2(V_m) \right] + \tau_{m,\text{min}},
\]

\[
\tau_h(V_m) = (\tau_{h,\text{max}} - \tau_{h,\text{min}}) \left[ 1 - h_l^2(V_m) \right] + \tau_{h,\text{min}}.
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\(V_m\) is the membrane potential in mV, \(E\) is the reversal potential, and all parameters in the argument of tanh functions have mV unit. Parameters for time constants are in ms. The maximal conductances for the generic model are shown in column \(g\) (in nS). Parameters of the leakage current are also shown (no voltage-dependence here). See text for additional definitions.
where parameters for the $m$ and $h$ functions are shown in Table 1. In addition to the leakage- and voltage-dependent membrane currents, we applied an additive intrinsic current noise term (brown noise, 3-pA peak-to-peak variations). Synaptic currents used under the second and third type of inputs were described using a first-order kinetics of transmitter release as:

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

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

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

The synaptic activation term depends on the presynaptic membrane potential as

$$S_a(V_{pre}) = \tanh \frac{V_{pre} - V_{thes}}{V_{slope}},$$

when $V_{pre} > V_{thes}$, otherwise $S_a(V_{pre}) = 0$. We used these equations and functions to simulate chemical synaptic currents both in the computational model and in the dynamic-clamp software.

RESULTS

First, we developed a simple computational model that reproduced many important physiological properties of BNST neurons as observed in our earlier current-clamp studies (Szücs et al. 2012). Such features include the input resistance, membrane time constants, depolarizing voltage sag, afterdepolarization, and others that characterize the voltage responses of BNST neurons under current step stimulation. Previous studies identified three main types of BNST neurons (Hammack et al. 2007; Hazra et al. 2011). Specifically, type I neurons are characterized by the presence of a depolarizing voltage sag indicative of their intrinsic hyperpolarization-activated cation current ($I_h$). Type II neurons, in addition to the $I_h$, express strong T-type Ca currents that promote their postinhibitory rebound firing. Type III neurons display more negative resting membrane potential than the other two types and also show inward rectification under negative current steps. Our generic model consists of several voltage-dependent currents that are present in these type of neurons. In particular, we include the hyperpolarization-activated cation current $I_{h}$, the inwardly rectifying $I_{KIR}$ current $I_{Kir}$, and the transient K current $I_{K}$.

Additionally, we include the persistent Na current $I_{NaP}$ (Vervaeke et al. 2006) and the M current (Lee and Kwag 2012) that produces prominent spike frequency adaptation commonly observed in the BNST neurons. While our model reproduces several physiological features and responses of the biological BNST neurons, our focus here is to analyze the effects of specific voltage-gated currents on the neuronal excitability and firing patterns in a more general setting.

Stimulus protocols to characterize the intrinsic excitability. Physiological properties of biological neurons are commonly studied in current-clamp experiments with rectangular current steps. In fact, the great majority of studies addressing intrinsic excitability of neurons in vitro utilize such stimulation as the primary experimental technique. The output of the generic model under DC step stimulation is shown in Fig. 1A. Here, 500-ms long current steps were injected into the model to evoke subthreshold voltage responses and firing at more depolarized levels. Current steps were gradually incremented by 10 pA from an initial level of $-200$ pA similarly to that in our prior current-clamp experiments with biological neurons (Szücs et al. 2010). Here, the action of the $I_h$ and $I_{Kir}$ is clearly recognized as depolarizing voltage sag under negative current levels as well as inward rectification. Moderate afterdepolarization following the termination of the current steps is associated with the deactivation of the $I_h$, and this phenomenon is also typical in many BNST neurons, especially in type I cells (Szücs et al. 2012).
The current step stimulation demonstrated here was selected as the first type input because it is considered as a standard tool to investigate the excitability of biological neurons. The I-O curves of the model neurons were obtained using higher resolution mapping of the firing responses than in Fig. 1A. Specifically, we used current levels incremented by 2.5 pA from an initial level of 0. Figure 1B demonstrates the spike number vs. current relationship for the generic model. Here, the neuron remains subthreshold up to +42.5 pA and then emits a gradually increasing number of spikes. The model was stimulated up to a current level that was three times of the rheobase (+130 pA). This was a general strategy in all simulations, i.e., I-O functions were surveyed in the range ending at three times the threshold stimulus intensity (rheobase) for the reference neuron.

The second type of stimulation used a mixture of excitatory and inhibitory synaptic conductances, which is a more realistic approximation to what biological neurons experience in natural conditions. The input waveforms used here were identical to those used in our prior dynamic-clamp studies with BNST neurons (Szücs et al. 2010). To calculate I-O functions of the model neurons under such input, the strength of the synaptic conductances was gradually increased in the successive sweeps. In particular, AMPA-, NMDA-, and GABA<sub>A</sub>-type synaptic conductances were incremented by 0.5, 1, and 1 nS, respectively. In effect, this protocol simulated a symmetrical upscaling of all synaptic connections to the neuron. As demonstrated in Fig. 1D, the number of spikes emitted by the model neuron was gradually increasing with the strength of the stimulation and an I-O curve analogous to the one in the DC experiment (Fig. 1B) was obtained. Here, the firing threshold of the input was <i>g<sub>AMPA</sub> = 5 nS</i> and the stimulation was continued up to three times of this level, 15 nS.

Using synaptic conductance as the control parameter of the stimulation is reasonable because biological neurons in natural conditions receive conductance, rather than current inputs. Also, the synaptic conductance in the simulations can be incremented in fine steps and its strength does not depend on the response of the neuron. However, one might suggest to use the synaptic current as the input parameter and analyze the firing responses as a function of the current. As shown above, the synaptic current (<i>I<sub>syn</sub></i>) depends on both the presynaptic and postsynaptic voltages; hence, the relationship between the synaptic conductance and the mean synaptic current cannot be described in a simple formula. In fact, obtaining the time average of the synaptic current for a given synaptic conductance requires the integration of the model during the entire stimulus sweep (5 s). Therefore, we implemented a two-step algorithm to use the synaptic current as the control parameter of the stimulation. First, we incremented the synaptic conductances in a wide range, integrated the model, and obtained the corresponding mean synaptic current values for each sweep. Next, we defined the stimulus intensity range similarly to that in the previous methods, i.e., from the <i>I<sub>syn</sub></i> threshold of firing to three times of that. The increment of the synaptic current was then set to 1/30 of the one to three times threshold intensity range. The stimulation of the model was then performed at 30 increasing levels of the synaptic current using <i>g<sub>AMPA</sub></i> values predicted by the previous sampling. Hence, the I-O curves obtained using such technique could be directly compared with those under the simple current step stimulation; in both cases the net input current was increased in a linear fashion. While the first type of stimulation uses the standard protocol for characterizing the intrinsic excitability of neurons, the second stimulation is designed to analyze firing under physiologically more realistic conditions. Unlike under DC step stimulation, the amount of current injected into the model varies rapidly and depends on the membrane potential of the neuron (as postsynaptic) when using the simulated synaptic bombardment (Fig. 1C, gray traces). Also important to note, the membrane potential under the synaptic type inputs fluctuates in a wider range than when using the DC steps. As an example, the membrane potential of the model neuron in Fig. 1A never visits levels below −53 mV whenever currents more positive than rheobase are injected. However, the membrane potential between spikes can reach −67 mV under the second type input. Clearly, the synaptic type input drives the neurons firing in a broader dynamic range than the current step stimulation.

**Differential effects on intrinsic excitability by manipulation of voltage-gated currents.** Following the development of the neuron model and the design of stimulus protocols, we performed a systematic analysis of firing by manipulating the intrinsic cellular properties and stimulating the model with the current steps and synaptic inputs. Here, the maximal conductance of a selected voltage-dependent current was adjusted in small steps (starting at zero) and I-O curves from the three types of experiments were calculated. The maximal conductances of the other nonvaried currents were fixed to the level of the generic model (as in Table 1). Maximal stimulus intensities were always set to three times of the threshold intensity of the reference model neuron (reference meaning that the target voltage-gated conductance was set to zero, not the same as the generic model). Hence, in each model experiment we surveyed the excitability profile of the neuron in a wide range spanning from inputs near spike threshold to those inducing more intense firing.

Figure 2 shows results from such simulations demonstrating the impact of the inward rectifying K<sub>ir</sub> current in this example. The voltage traces in Fig. 2A illustrate the response of the model without <i>I<sub>Kir</sub></i> (<i>g<sub>Kir</sub> = 0 nS</i>, reference model) at two selected current levels. The voltage trace under the −200-pA level shows prominent depolarizing voltage sag and afterdepolarization due to the action of the <i>I<sub>Kr</sub></i> that is present in the model. The firing at the +60-pA level is almost periodic; however, slight spike frequency adaptation is noticed due to the action of the intrinsic M current. The behavior of this model neuron is similar to type I BNST neurons (Szücs et al. 2012). Introducing the <i>I<sub>Kir</sub></i> at the 20-nS level (maximal strength) changes the voltage response markedly as shown in Fig. 1B. The input resistance is reduced and the spiking at +60 pA is significantly delayed relative to the onset of the current. This behavior is typical in type III BNST neurons (Hammack et al. 2007; Rodriguez-Sierra et al. 2013), and it reflects the action of the slowly activating K<sub>ir</sub> current during depolarizing current steps. I-O functions of the model neuron with increasing amount of K<sub>ir</sub> current are shown in Fig. 2, C–E. With the use of the current step stimulation (Fig. 2C), a gradual shift of the I-O curve is observed; the rheobase increases by 20% from the initial value of +40 pA when the K<sub>ir</sub> is not included in the model. This indicates the K<sub>ir</sub> current mediated reduction of excitability under the standard current step stimulation. The same analysis for the synaptic type input shows a stronger impact of K<sub>ir</sub> upregulation; the threshold AMPA
conductance of the Kir current shifts the spike threshold to the right. Here, firing in the reference model neuron is reduced and the onset of firing is delayed due to the slow deactivation of $I_{\text{Kir}}$. C: family of I-O curves under the current step stimulation. The leftmost black curve is obtained from the model without $I_{\text{Kir}}$ (reference model). D and E: similar to C, but here spike numbers are shown as a function of the AMPA conductance ($g_{\text{AMPA}}$) or the mean total synaptic current ($I_{\text{Syn}}$) under the synaptic type stimulation. Increasing the maximal conductance of the $I_{\text{Kir}}$ current shifts the spike threshold to the right in each model experiment. The strongest effect is observed under the synaptic type input ($E$) when the mean total synaptic current is used as control parameter.

While the threshold stimulus intensities (“rheobase levels”) can be used to quantify changes of intrinsic excitability due to the action of voltage-gated currents, we preferred using a parameter that characterized the I-O response in the entire range of stimulus intensities. Also, comparing data from experiments involving different stimulus protocols and with different number of spikes required a way of normalizing the data. We utilized the following method: the total number of spikes emitted in each model experiment. The strongest effect is observed under the synaptic type input ($E$) when the mean total synaptic current is used as control parameter.

The summary of all these simulations is shown in Fig. 3 for five different voltage-gated currents. We gradually increased the maximal conductances of the selected currents (21 levels for each), calculated the I-O curves of the model under the two types of stimulations ($I_{\text{DC}}$, $g_{\text{AMPA}}$, and $I_{\text{Syn}}$ as control parameters), and calculated the normalized cumulative spike numbers. Figure 3, A–C, top, demonstrates the actions of hyperpolarizing membrane currents that tend to decrease intrinsic excitability. Indeed, all three plots show the decrease of the normalized spike numbers, but we find strong differences in the trajectories. As an example, the inward rectifier at maximal strength increases the excitability of the model neuron in an input-dependent manner. Here, the model was subjected to 2 types of stimulation while the maximal conductance of the $I_{\text{Kir}}$ was adjusted between 0 and 20 nS in 1-nS increments. A: response of the model with no intrinsic $I_{\text{Kir}}$ at 2 selected current levels (−200 and +60 pA). B: voltage traces at the same levels of injected current and with 20 of nS $I_{\text{Kir}}$ added. The input resistance of the latter model neuron is reduced and the onset of firing is delayed due to the slow deactivation of $I_{\text{Kir}}$. C: family of I-O curves under the current step stimulation. The leftmost black curve is obtained from the model without $I_{\text{Kir}}$ (reference model). D and E: similar to C, but here spike numbers are shown as a function of the AMPA conductance ($g_{\text{AMPA}}$) or the mean total synaptic current ($I_{\text{Syn}}$) under the synaptic type stimulation. Increasing the maximal conductance of the $I_{\text{Kir}}$ current shifts the spike threshold to the right in each model experiment. The strongest effect is observed under the synaptic type input ($E$) when the mean total synaptic current is used as control parameter.

Fig. 2. Upregulation of the inward rectifying $K_\text{ir}$ ($I_{\text{Kir}}$) current decreases the excitability of the model neuron in an input-dependent manner. Here, the model was subjected to 2 types of stimulation while the maximal conductance of the $I_{\text{Kir}}$ was adjusted between 0 and 20 nS in 1-nS increments. A: response of the model with no intrinsic $I_{\text{Kir}}$, at 2 selected current levels (−200 and +60 pA). B: voltage traces at the same levels of injected current and with 20 of nS $I_{\text{Kir}}$ added. The input resistance of the latter model neuron is reduced and the onset of firing is delayed due to the slow deactivation of $I_{\text{Kir}}$. C: family of I-O curves under the current step stimulation. The leftmost black curve is obtained from the model without $I_{\text{Kir}}$ (reference model). D and E: similar to C, but here spike numbers are shown as a function of the AMPA conductance ($g_{\text{AMPA}}$) or the mean total synaptic current ($I_{\text{Syn}}$) under the synaptic type stimulation. Increasing the maximal conductance of the $I_{\text{Kir}}$ current shifts the spike threshold to the right in each model experiment. The strongest effect is observed under the synaptic type input ($E$) when the mean total synaptic current is used as control parameter.
synaptic input as stimulation. Lastly, the persistent Na current has a strong impact in the excitability and the normalized spike numbers grow steeply as $g_{NaP}$ is increased. Similarly to the $M$ current, the persistent Na current has a stronger effect on the firing response when the current step stimulation is used than when the synaptic input ($g_{AMPA}$ as control parameter). We note that when the synaptic current is used as control parameter, the model becomes overexcited at conductance levels above $g_{NaP} = 3.5$ nS and spike generation becomes erratic. In such simulations, I-O curves could not be accurately obtained between threshold $I_{syn}$ and three times of that level (star symbol in Fig. 3E).

Figure 3, A–E, bottom panels demonstrate the same data in a different format. Here the excitability profiles of the synaptic input using either $g_{AMPA}$ or $I_{syn}$ are compared with those of the DC step stimulation. Relative effects are calculated as the ratio between the observed excitability changes under synaptic inputs relative to that under the current step stimulation. As an example, the addition of the $K_v$ current causes $\sim 2$ times greater reduction of firing under synaptic type input than under current steps ($F$; open symbols, $g_{AMPA}$ as control parameter). The addition of the $M$ ($H$) or $Na_P$ current ($J$), however, has a weaker effect under the synaptic type input than under the DC stimulation.

Fig. 3. Impact of voltage-gated membrane currents is sensitive to the type of stimulation used to evoke firing responses. In each plot from A–E, the normalized cumulative spike number is plotted against the maximal conductance of a selected membrane current (top). The addition of the $I_{Kir}$, $I_A$, and $I_M$ currents decreases the observed excitability while the action of the $h$ and $Na_P$ currents is the opposite. The impact of the $K_v$ current is the greatest on the synaptic type input when the mean total synaptic current is used as control parameter (A; normalized spike number drops to 21.9% at 20 nS $g_{Kir}$). At the same time, the $M$ current reduces firing the most effectively when using the current step protocol (C; 28% at 10 nS $g_{AMPA}$). The addition of $h$ current has a moderate effect on the excitability (D), but the $I_{NaP}$ is far more effective (E). The star symbol in E indicates a region when the model receives too strong excitation and I-O curves are not obtained (see text). Bottom: ratio between the observed excitability changes under synaptic inputs relative to that under the current step stimulation. As an example, the addition of the $K_v$ current causes $\sim 2$ times greater reduction of firing under synaptic type input than under current steps (F; open symbols, $g_{AMPA}$ as control parameter). The addition of the $M$ ($H$) or $Na_P$ current ($J$), however, has a weaker effect under the synaptic type input than under the DC stimulation.
conductance of a specific voltage-gated membrane current regulate synaptic responses either more effectively or less, \( I_M \) and \( I_{NaP} \) than the firing of the same neuron under current step stimulation. Therefore, the impact of specific voltage-gated currents on intrinsic excitability is sensitive to the temporal structure of the input.

We note that the intrinsic noise (3 pA) in the model has a small but detectable impact on the number of spikes that are emitted during repeated sweeps of the same stimulus. Hence, the I-O curves obtained under such input are not perfectly identical; spike numbers differ by ±1 in various locations along the curve. However, the coefficient of variation (mean ± SD) of the cumulative spike numbers is found to be very small for each stimulus protocol (<0.4% for all 3 conditions). Hence, the standard errors of relative cumulative spike numbers are negligible compared with the observed differences across stimulus conditions.

**Dynamic-clamp study of neuronal excitability with insertion of a synthetic \( K_r \) current.** The computational approach allows us to adjust the intrinsic biophysical properties of the model neuron typically not achievable in experiments with biological neurons. Indeed, accurate manipulation of a selected voltage-gated membrane current is challenging using pharmacological tools, especially when rapid and reversible upregulation is the aim. At the same time, the dynamic-clamp technique allows us to insert a synthetic voltage-dependent membrane current into the biological neuron (Tateno and Robinson 2011; Vervaeke et al. 2006) while performing the same type of experiments as done with the computational model above. Hence, in the next set of experiments we used BNST neurons in brain slices and the dynamic clamp to verify some of the predictions from the computational study. For these experiments, the BNST neurons were isolated from their endogenous synaptic inputs using a combination of blockers of glutamatergic and GABAergic transmission. We chose the inward rectifier as the target conductance, because its kinetics (first-order activation and no inactivation) was simple compared with the others and our abundant earlier data from type III BNST neurons allowed us to carefully tune the model parameters of the synthetic \( K_r \) current. We performed such hybrid experiments on nine BNST neurons that were classified as either type I or type II cells. Such neurons have weak or no intrinsic \( K_r \) currents, and insertion of a synthetic \( K_r \) current would expectedly alter their dynamics in a way that they become more similar to type III neurons that posses strong endogenous \( K_r \) currents (Hammack et al. 2007; Szücs et al. 2012). Indeed, this is what we found in all experiments. Figure 4 demonstrates results from such experiments. In this example, a type II neuron exhibited strong voltage sag and postinhibitory rebound firing during the standard DC step stimulation (Fig. 4A, no \( I_{K_r} \) injected). Insertion of an 8-nS synthetic \( K_r \) current decreased its input resistance, reduced the afterdepolarization, and eliminated the postinhibitory firing. The spike number vs. current relationship for this pair of experiment is shown in Fig. 4C. As expected, the insertion of the synthetic \( I_{K_r} \) shifted the I-O curve to the right and decreased the cumulative spike number. Next, similarly to that in the computational model, we performed stimulation with simulated synaptic bombardment and varied the amount...
of the synthetic $K_{ir}$ current (using 3 levels: 0, 5, and 8 nS). Again, we observed the shift in the I-O relationship and the effect of the $K_{ir}$ insertion was stronger in this type of experiment than under the DC step stimulation (Fig. 4D). Figure 4E shows the pooled normalized cumulative spike numbers for the three conductance levels used. In all biological experiments, the impact of $I_{Kir}$ was found to be stronger in the synaptic responses than in the firing under constant current step protocols, an observation that agrees well with the data from the model runs. Average normalized cumulative spike numbers for the current step and synaptic stimulation were 82.8 ± 3.4 vs. 54.0 ± 3.7% ($g_{Kir} = 5$ nS) and 74.3 ± 5.8 vs. 34.9 ± 5.4% ($g_{Kir} = 8$ nS), respectively ($n = 9; P < 0.0002$ for both, paired t-test). $K_{ir}$-induced changes of five physiological parameters measured under current step stimulation are summarized in Table 2.

**Analysis of membrane potential dynamics.** As mentioned above, the membrane potential of the neuron visits different regimes of voltage that, in principle, can explain the observed differences in the neuronal excitability. To analyze this claim we calculated three parameters to characterize the history of membrane potential under the stimulation. The first of such parameters was the spike threshold defined as the membrane potential level when its derivative reached +10 mV/ms. Using the arrival time of spike threshold as reference, we defined a 100-ms window preceding each spike. Next, we determined the most hyperpolarized $V_m$ level in each of such windows and also calculated the mean membrane potential. To differentiate between spikes evoked at different levels of stimulus intensity we used color mapping as shown in Fig. 5. Each point in Fig. 5, A–F, corresponds to a spike fired at a stimulus intensity indicated by its color (from blue toward red). As quickly noticed, points are more scattered along the $Y$ (voltage) scale for the synaptic type input than the ones under the current step stimulation. Hence, the spike threshold and mean and minimum voltage level parameters are more variable for the dynamic stimulation than those for the DC step stimulation. Furthermore, the mean voltage level under the DC step stimulation is ≈10 mV more depolarized than when the synaptic type input is used. Plots of the mean voltage level (Fig. 5B) and minimum voltage (Fig. 5C) for the current step stimulation show a prominent jump from $t = 0$ to $t = 100$ ms. This is due to the way these parameters are calculated, i.e., by using a 100-ms window before each spike. For the first spikes in the DC step sweeps, the 100-ms window contains part of the $V_m$ waveform when the injected current steps from 0 to +40 pA or more positive levels (see Fig. 1A). Beyond 100 ms all three parameters level out and show little variations across spikes. Figure 5, G–I, depicts the averaged values and standard errors for these three parameters as a function of the relative stimulus intensity (from 1 to 3 times threshold intensity). For both type inputs, the spike voltage threshold (Fig. 5G) declines to more negative levels as the stimulus intensity increases while the other parameters tend to move in the opposite direction (Fig. 5, H and I). We find the strongest differences between the two types of inputs when evaluating the mean and minimum voltage level parameters at medium and stronger intensity levels. Here, the two trajectories clearly diverge; the parameters for the current step input (black symbols) are consistently more positive than the ones for the synaptic type input (open square symbols). We performed two-sample Kolmogorov-Smirnov tests to compare the distributions of the three parameters at increasing levels of relative stimulus intensity. Very significant differences in the distributions were observed at relative intensities >1.5 for each voltage parameter (Fig. 5, G–I).

**Simulating homeostatic coregulation of voltage-gated currents.** The predictions from the computational model and the confirming results from the biological neurons suggested an interesting possibility that is related to the intrinsic excitability of neurons that undergo homeostatic regulation of multiple voltage-gated membrane conductances. In fact, upregulation of a specific voltage-dependent membrane current often involves changes in another current (MacLean et al. 2003; Marder and Goaillard 2006; Pratt and Aizenman 2007), so analyzing changes of intrinsic excitability in relation to more than one intrinsic currents is justified. The problem can be illustrated by comparing the input-dependent effects of one depolarizing and one hyperpolarizing membrane current. As we have shown, upregulation of the $K_{ir}$ current reduces firing under synaptic type input more effectively than under DC current steps (Figs. 3A and 4E), while the action of the $NaP$ current is the opposite (Fig. 3E). If the inward rectifier $I_{Kir}$ and the $NaP$ current are both upregulated in the neuron, they would balance each other and the net change in the intrinsic excitability would be expected to be minor. In the simulations of Fig. 6 we studied this scenario. Using the data from Fig. 3 we determined that a 30% decrease of excitability as caused by 20-nS $I_{Kir}$ in the model could be offset by the insertion of 2.5-nS $I_{NaP}$ when using the DC step stimulation. Figure 6A shows the voltage response of the generic model and the one with coregulated currents (both $I_{Kir}$ and $I_{NaP}$ up) at the +85-pA current level that represents the middle point in the I-O curve. The action of the $K_{ir}$ current is clearly visible as the delaying of firing under the depolarizing step. At the same time, the depolarizing effect of the $I_{NaP}$ increases the frequency of firing after the latency period, so the total number of spikes in the two conditions remain the same. As in the previous simulations, we surveyed the responses up to three times of the rheobase and calculated the I-O curve. Here, we observe a slight change in the shape of the curve but the cumulative spike number is barely affected (+0.4%) by the concurrent upregulation of the two currents. Nevertheless, comparison of data from the model experiments using the synaptic type of stimulation reveals a significant decrease of excitability (Fig. 6, C and D). In fact, the cumu-

**Table 2. Insertion of the synthetic $K_{ir}$ conductance alters the physiological parameters of BNST neurons measured in DC step experiments**

<table>
<thead>
<tr>
<th>$g_{Kir}$ (nS)</th>
<th>$V_{rheobase}$ (mV)</th>
<th>$R_{rheobase}$ (%)</th>
<th>$R_{-200}$ (%)</th>
<th>$Rheobase$,</th>
<th>Gain, (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-5.3 ± 0.5</td>
<td>87.1 ± 3.9</td>
<td>58.4 ± 2.8</td>
<td>59 ± 1.5</td>
<td>98.4 ± 5.3</td>
</tr>
<tr>
<td>5</td>
<td>-4.0 ± 0.7</td>
<td>71.2 ± 4.5</td>
<td>46.1 ± 2.9</td>
<td>10.4 ± 2.1</td>
<td>104.1 ± 7.6</td>
</tr>
</tbody>
</table>

Mean relative values (percentages) are shown for the $R_{rheobase}$, $R_{-200}$, and the gain parameters and mean absolute differences (shifts) are shown for the $V_{rheobase}$ and rheobase. $R_{-200}$ is the membrane resistance measured under the injection of −200 pA current while $R_{rheobase}$ is the extrapolarized resistance at resting membrane potential. BNST, bed nucleus of stria terminals (BNST). All parameters except the gain change very significantly due to $K_{ir}$ insertion ($P < 0.001$).
relative spike number measured from the responses of the co-regulated model drops to 60.1% relative to that of the generic model as a consequence of the strong rightward shift of the I-O curve. Hence, the results from the current step experiments suggest that the two neurons have similar excitability, while analysis of their synaptic responses allows a very different interpretation.

The $I_{\text{Kir}}$ and $I_{\text{NaP}}$ currents selected for these simulations have different voltage dependence, because the slopes of their steady-state activation curves are the opposite. It might be suggested that pairing depolarizing and hyperpolarizing membrane currents with similar voltage dependence of activation would produce less contrasting effects in the static vs. dynamic excitability of the neurons. We tested this possibility by pairing and upregulating all the other voltage-gated currents analyzed in our study. There were a total of six combinations: the two depolarizing currents ($I_{\text{NaP}}$ and $I_h$) were paired with the three K currents. We followed the same strategy as done with the $K_{\text{ir}}$-$\text{NaP}$ pair: we increased the maximal conductance of both the depolarizing and hyperpolarizing current in a way that the cumulative spike number under static stimulation remained almost exactly the same as in the generic model (differed by $<0.5\%$). Then, using these maximal conductance values, we ran the simulation with the synaptic type input and calculated the cumulative spike numbers. The relative cumulative spike numbers from such simulations were as follows. $I_{\text{Kir}}$-$I_{\text{NaP}}$: 60.1% (as shown); $I_{\text{A}}$-$I_{\text{NaP}}$: 82.6%; $I_{\text{M}}$-$I_{\text{NaP}}$: 103.9%; $I_{\text{Kir}}$-$h$: 99.6%; $I_{\text{A}}$-$h$: 102.6%; and $I_{\text{M}}$-$h$: 104.3%. These results show that the coregulation of the $I_{\text{Kir}}$ and $I_{\text{NaP}}$ currents produces the most contrasting changes in the static vs. synaptic responses of the neuron. Pairing the transient K-current $I_{\text{A}}$ and the $I_{\text{NaP}}$ appears to work better in balancing the synaptic responses. Here, the relative cumulative spike number drops by 17.4% in the coregulated model, much less than in the case of the $I_{\text{Kir}}$-$I_{\text{NaP}}$ pair. The remaining combinations of currents are all appropriate for homeostatic regulation of both static excitability and synaptic responses. However, we note that increasing the maximal conductance of the h current from 2 nS in the generic model to 4 nS in the upregulated model causes only modest change in the excitability (see Fig. 3D) that can be compensated by a slight upregulation of any of the hyperpolarizing K currents.

Fig. 5. Analysis of membrane potential ($V_m$) time series of the generic model neuron. A–F: spike threshold, the mean voltage level, and the minimum voltage-level parameters (as columns) for the 2 types of stimulation (as rows). All parameters are plotted against stimulus time. Each point in the graphs corresponds to a spike, and its color indicates the stimulus intensity (navy blue is at threshold, and red is at 3 times threshold). Averages of the above 3 parameters are plotted against the relative stimulus intensity in G–I. The spike threshold and mean and minimum voltage parameters calculated for the current step stimulation (black symbols) are more positive than the same parameters for the synaptic type input in the entire range of relative stimulus intensity. Asymptotic $P$ values calculated in two-sample Kolmogorov-Smirnov tests ($P_{\text{K-S}}$) are shown for each parameter. They are well below $P = 0.05$ at most stimulus intensities.
In this study we analyzed the firing responses of biological and model neurons using static current stimuli and under the action of simulated synaptic inputs. We manipulated specific voltage-dependent membrane currents by altering their maximal conductances that simulated the action of plastic or homeostatic regulation of intrinsic cellular properties. Under such manipulation, the neurons’ excitability was altered in the anticipated directions, but the magnitude of such changes strongly depended on the type of input used to stimulate the neuron.

The voltage-dependent conductances we surveyed in this study have been described as playing important regulatory role in setting the firing pattern of neurons of the extended amygdala but also in a number of other brain areas. A-type K channels containing subunits of the Kv4 and Kv1 families regulate the repetitive firing and spike timing in cortical neurons (Carrasquillo et al. 2012; Hyun et al. 2013; Jung and Hoffman 2009). They have been shown to undergo activity-dependent changes that manifest as a form of long-term potentiation of intrinsic excitability (Francesconi et al. 2009; Hyun et al. 2013), so they can contribute to the maintenance of excitatory-inhibitory balance in neural circuits (Campanac et al. 2013). Inward rectifying K channels also delay the spike responses of neurons under depolarizing inputs, but their role is more significant in setting the resting membrane potential and input resistance of cells (Young et al. 2009). At the same time, the effects of transient and persistent Na channels on excitability are more convoluted and even paradoxical effects have been reported (Kispersky et al. 2012; Vervaeke et al. 2006). In fact, the action of any of the voltage-dependent membrane currents depends on others that concurrently activate or inactivate during the operation of the neuron.

As a common and well-accepted approach, experimenterers studying plastic regulation of the intrinsic excitability of neurons apply incrementing levels of intracellular current and measure the output firing rate of the neuron (Carrasquillo et al. 2012; Malik and Chattarji 2012; Poolos et al. 2002). These experiments provide several quantitative measures of the neurons’ excitability such as the rheobase and gain. Changes in excitability of a neuron are detected when the intercept or slope of the I-O curve is altered. The first case is commonly referred to as an additive change of excitability, while the second is gain modulation (Chance et al. 2002; Thurley et al. 2008). In our model simulations and experiments, the upregulation of specific voltage-dependent currents has a stronger effect on the rheobase than on the gain (Naude et al. 2012). In addition to the threshold intensity and gain, we calculated the cumulative spike number to characterize the neuronal firing in a more broad range of inputs, i.e., in the one to three times threshold intensity range. Normalizing the cumulative spike numbers to the value observed in the reference neuron allowed us to compare the impact of specific voltage-gated currents on the excitability even when different types of inputs were used. The relative cumulative spike numbers strongly depended on the type of input used to elicit the firing of the neuron. However, one can also notice that limiting the intensity range to one to two times threshold in the calculations would have resulted in even more diverging trajectories for the two stimulus protocols. This is because the I-O functions display additive shifts rather than gain modulation of excitability under the upregulation of the currents.

Comparing the relative effect of the different voltage-gated currents (Fig. 3), we notice a relationship between their efficiency in shaping the firing and their voltage-dependence. The inward rectifier $I_{K_{ir}}$ and the $h$ current are both activated with hyperpolarization; they do not inactivate and are partially active at the resting membrane potential. The impact of these two currents is approximately two times greater under the synaptic type input than when using the static current stimulation ($g_{AMPA}$ vs. current steps comparison). In contrast, the M current and the persistent Na current activate with depolarization and they are almost entirely deactivated at rest. The relative impact of these currents under the synaptic type inputs

**DISCUSSION**

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is only half of that measured under DC steps. Examining the mean levels and fluctuations of voltage responses under the different type of stimuli can help explaining these differential effects. Both in the model and biological neurons, firing in response to constant current steps starts at relatively depolarized levels and the membrane potential remains in the region more positive than −50 mV during the current step. Hence, the relative contribution of hyperpolarization-activated currents ($I_{\text{h}}$) is diminishing during the depolarizing step and the firing is more influenced by the action of currents typically activate in this region (i.e., the $I_{\text{NaP}}$ and $I_{\text{N}}$). Thus depolarization-activated currents play a more dominant role in shaping the firing pattern under DC steps. However, when a mixture of excitatory and inhibitory synaptic inputs is used as stimulus, the membrane potential often visits levels below −60 mV and the hyperpolarization-activated currents have a far stronger impact on the firing. They do not deactivate as much as during a maintained depolarization with DC steps.

Considering the above, analyzing I-O functions of neurons under DC step stimulation alone one might either over- or underestimate the impact of specific voltage-gated channels in regulating the intrinsic excitability of neurons. The problem becomes even more complex when more than one voltage-dependent currents are up- or downregulated by homeostatic processes. Such mechanisms have been demonstrated in a number of nervous systems, and they often involve concurrent upregulation of one depolarizing and one hyperpolarizing membrane conductance (MacLean et al. 2003; Marder and Goaillard 2006; Pratt and Aizenman 2007; Turrigiano 2011). As an example, if the excitability of the neuron was to increase to a level that is functionally not desired, the action of the upregulated hyperpolarizing current would bring the excitability back to the optimal range. Our results suggest that even when the intrinsic excitability of the neurons under DC steps stimulation appears to remain unaffected by the homeostatic changes in two membrane currents, the firing under synaptic inputs can change dramatically. This occurs when the voltage dependence of activation of the inward and outward currents is markedly different, as in our case with the $K_\text{ir}$ and $N_\text{aP}$ currents. Paradoxical or antihomeostatic effects of upregulation of Na channels have been demonstrated both in biological neurons and computational models when analyzing their excitability under static stimuli (Kispersky et al. 2012; Vervaeke et al. 2006). Our present findings also demonstrate contrasting effects of intrinsic membrane currents on the firing output of neurons. Nevertheless, we showed that these observations can be well explained in the context of voltage dependence of activation of the currents.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: A.S. conception and design of research; A.S. performed experiments; A.S. and R.H. analyzed data; A.S. and R.H. interpreted results of experiments; A.S. prepared figures; A.S. and R.H. drafted manuscript; A.S. edited and revised manuscript; A.S. approved final version of manuscript.

REFERENCES


