

Relative Timescale of Channel Voltage-Dependence and Channel Density Regulation Impacts Assembly and Recovery of Activity

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eLife Assessment

This **important** computational study investigates homeostatic plasticity mechanisms that neurons may employ to achieve and maintain stable target activity patterns. The work extends previous analyses of calcium-dependent homeostatic mechanisms based on ion channel density by considering activity-dependent shifts in channel activation and inactivation properties that operate on faster and potentially variable timescales. The model simulations provide **solid** evidence for the potential functional importance of these mechanisms.

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Abstract

Neurons can maintain stable activity profiles over their lifetimes despite ion channel turnover over minutes to weeks. Neuronal activity is also influenced by regulating the voltage-dependence of ion channels. How do these two forms of plasticity work together to maintain a stable activity profile? We augment a classical model of activity-dependent ion channel density regulation with a mechanism that adjusts channel voltage-dependence based on activity. These findings reveal that the timescale of these mechanisms shape the specific electrical activity patterns that achieve a target activity profile. Moreover, alterations in channel voltage-dependence can impact a neuron's ability to recover from perturbations. These results highlight a potentially distinct role for activity-dependent regulation of channel voltage-dependence in maintaining stable neuronal activity profiles.

Introduction

Neurons maintain stable activity patterns over years despite continual turnover in their ion channel composition, which occurs on timescales from days to weeks. Experimental studies have shown that neurons homeostatically regulate both channel number (Desai et al 1999 , Haedo &

Golowasch 2006 [\[1\]](#), Mizrahi et al 2001 [\[2\]](#), Santin & Schulz 2019 [\[3\]](#), Thoby-Brisson & Simmers 2002 [\[4\]](#), Turrigiano et al 1994 [\[5\]](#), Turrigiano et al 1995 [\[6\]](#), Viteri & Schulz 2023 [\[7\]](#)) and channel voltage-dependence (Gasselín et al 2015 [\[8\]](#), Haedo & Golowasch 2006 [\[9\]](#), Thoby-Brisson & Simmers 2002 [\[4\]](#)) to stabilize their activity.

These mechanisms have been widely studied through computational modeling, but most of this work has focused on changes in channel number (Abbott & LeMasson 1993 [\[10\]](#), Alonso et al 2023 [\[11\]](#), Golowasch et al 1999 [\[12\]](#), Gorur-Shandilya et al 2020 [\[13\]](#), LeMasson et al 1993 [\[14\]](#), O’Leary et al 2014 [\[15\]](#), Siegel et al 1994 [\[16\]](#), Srikanth & Narayanan 2015 [\[17\]](#)). This is largely due to the plethora of work indicating such changes occur (Desai et al 1999 [\[18\]](#), Haedo & Golowasch 2006 [\[9\]](#), Mizrahi et al 2001 [\[2\]](#), Santin & Schulz 2019 [\[3\]](#), Thoby-Brisson & Simmers 2002 [\[4\]](#), Turrigiano et al 1994 [\[5\]](#), Turrigiano et al 1995 [\[6\]](#), Viteri & Schulz 2023 [\[7\]](#)). However, this emphasis also reflects the challenges associated with precisely measuring changes in channel voltage-dependence. Inter-neuronal variability in activation and inactivation curves can obscure clear patterns of activity-mediated modifications, especially when analyzed at the population level (Turrigiano et al 1995 [\[6\]](#)). As a result, our understanding of how modifications to channel voltage-dependence contributes to activity stability remains limited.

This gap is important to address given the ubiquity of channel voltage-dependence regulation. Neurons modulate voltage-dependence through mechanisms ranging from rapid post-translational modifications (Fraser & Scott 1999 [\[19\]](#), Johnson et al 1994 [\[20\]](#)) or slower processes like subunit synthesis and degradation (Wu et al 2023 [\[21\]](#)). These processes are known to be associated with calcium-mediated second messengers. Intracellular calcium is also thought to play an important role in maintaining neuronal activity set points (LeMasson et al 1993 [\[14\]](#), Turrigiano et al 1994 [\[5\]](#)). As such, calcium-mediated homeostasis may operate, at least in part, by tuning channel voltage-dependence—yet the consequences of this form of regulation are largely uncharacterized.

To address this, we extended a state-of-the-art computational homeostatic model. This model stabilizes neuronal activity by monitoring intracellular Ca^{2+} concentrations and inserting and deleting ion channels (Alonso et al 2023 [\[11\]](#)). To this model, we added a mechanism that alters channel voltage-dependence. Importantly, this model enabled independent control over the timescales of channel number and channel voltage-dependence alterations. This organization of the model let us simulate fast changes in voltage-dependence, such as those driven by phosphorylation, or slower changes, like those resulting from synthesis or degradation of channel subunits. Our central finding is that the timescale of voltage-dependence regulation influences the specific channel configurations a neuron adopts—both while attaining a prespecified activity target and while maintaining it during perturbation.

Results

The Model

To explore how modifications in channel voltage-dependence influence a neuron’s capacity to achieve an activity profile as changes in ion channel density occur, we created a computational model. This model was built around a single compartment, Hodgkin-Huxley type conductance-based neuron with seven intrinsic currents (see Turrigiano et al (1995) [\[6\]](#) or Box 1, Supplement 1 [\[22\]](#)). The neuron model consisted of a fast Na^+ current, two Ca^{2+} currents, three K^+ currents, a hyperpolarization-activated inward current (I_h), and a leak current. Each current was described by its maximal conductance (number of ion channels) and activation curves. Some currents also possessed inactivation curves as well. The (in)activation curves described the effects of voltage on the opening and closing of the membrane channels.

The activity-dependent regulation of channel number and (in)activation curves employed a strategy that uses intracellular Ca^{2+} concentrations as a proxy for the neuron's activity (Abbott & LeMasson 1993 [↗](#), Alonso et al 2023 [↗](#), Golowasch et al 1999 [↗](#), Gorur-Shandilya et al 2020 [↗](#), LeMasson et al 1993 [↗](#), O'Leary et al 2014 [↗](#), Siegel et al 1994 [↗](#), Srikanth & Narayanan 2015 [↗](#)). Specifically, we followed the approach used in Liu et al (1998) [↗](#) and Alonso et al (2023) [↗](#), for altering channel density to guide our approach to altering (in)activation curves. In those models, the intracellular Ca^{2+} concentration and three filters (also called sensors) of the Ca^{2+} concentration were computed. The three sensors were employed to better differentiate between the activity of tonic spiking and bursting neurons (Alonso et al 2023 [↗](#), Liu et al 1998 [↗](#)). A target activity profile was chosen by prespecifying the target values of these sensors. These values were selected through trial and error to consistently produce regular bursting across a specific range of initial conditions (see Methods). When deviations from these Ca^{2+} sensor targets were detected, channel densities were altered to up or down regulate the number of channels in the membrane (Alonso et al 2023 [↗](#), Liu et al 1998 [↗](#)). Details on how the sensors were computed and how the target activity—defined by the target sensor values—was specified are provided in the Methods.

We expanded on these models by incorporating a mechanism that shifts the (in)activation curves in response to deviations from the Ca^{2+} sensor targets. This was accomplished by changing the half-(in)activation voltages of the (in)activation curves, mirroring strategies employed in Liu et al (1998) [↗](#) and Alonso et al (2023) [↗](#). The details of the Model, with their equations and assumptions are described in the Methods section. A brief overview is provided in Box 1 [↗](#).

Importantly, the response times of the mechanisms responsible for ion channel insertion and deletion, as well as those for shifts in (in)activation curves, were independently controlled. In the model, these response times were initially set two orders of magnitude apart. This reflected the slower processes of protein synthesis and degradation associated with channel insertion and deletion. This contrasted with faster time scales of post-translational modifications such as channel phosphorylation. Specifically, the timescale for changes in maximal conductance was set at $\tau_g = 600$ s, and the timescale for half-(in)activation shifts at $\tau_{half} = 6$ s. Later, we adjusted the response times of the (in)activation curve shifts. In prior computational studies of activity-dependent regulation, τ_g was set to be less than 10 seconds, substantially faster than the timescales used in our model (Alonso et al 2023 [↗](#), Liu et al 1998 [↗](#)). This study lengthens this timescale and explores the impact of varying the relative timescales of maximal conductance changes (τ_g) and half-(in)activation shifts (τ_{half}).

Bursters Assembled by Activity-Dependent Alterations in Ion Channel Density and Channel Voltage-Dependence

Figure 1 [↗](#) shows the full model, with the two regulation mechanisms in operation. From a set of starting randomly chosen parameters (maximal conductances and half- (in)activations), referred to as Starting Parameters 1 (SP1), the activity-dependent regulation mechanism altered the ion channel density and voltage-dependences to assemble an electrical activity pattern that satisfied the Ca^{2+} targets. The targets were chosen so that a burster self-assembled (see Methods). The final ion channel density and voltage-dependences are shown in the bar graphs (**Figure 1A** [↗](#)). The first two rows in **Figure 1A** [↗](#) show the electrical activity of the neuron as the model seeks to satisfy the Ca^{2+} targets. As it does, the model assembled a burster. The second row shows the activity over the entire trace and the first row shows blows up at: the start (a), about 100 minutes (b) and at the end of the simulation (c). At the time shown in (b), the neuron was bursting, but the Ca^{2+} targets had not been achieved. There were two indications that this burster did not satisfy the specified Ca^{2+} targets: (1) the model still continued to adjust the properties of the ion channel repertoire here and (2) a was not yet zero.

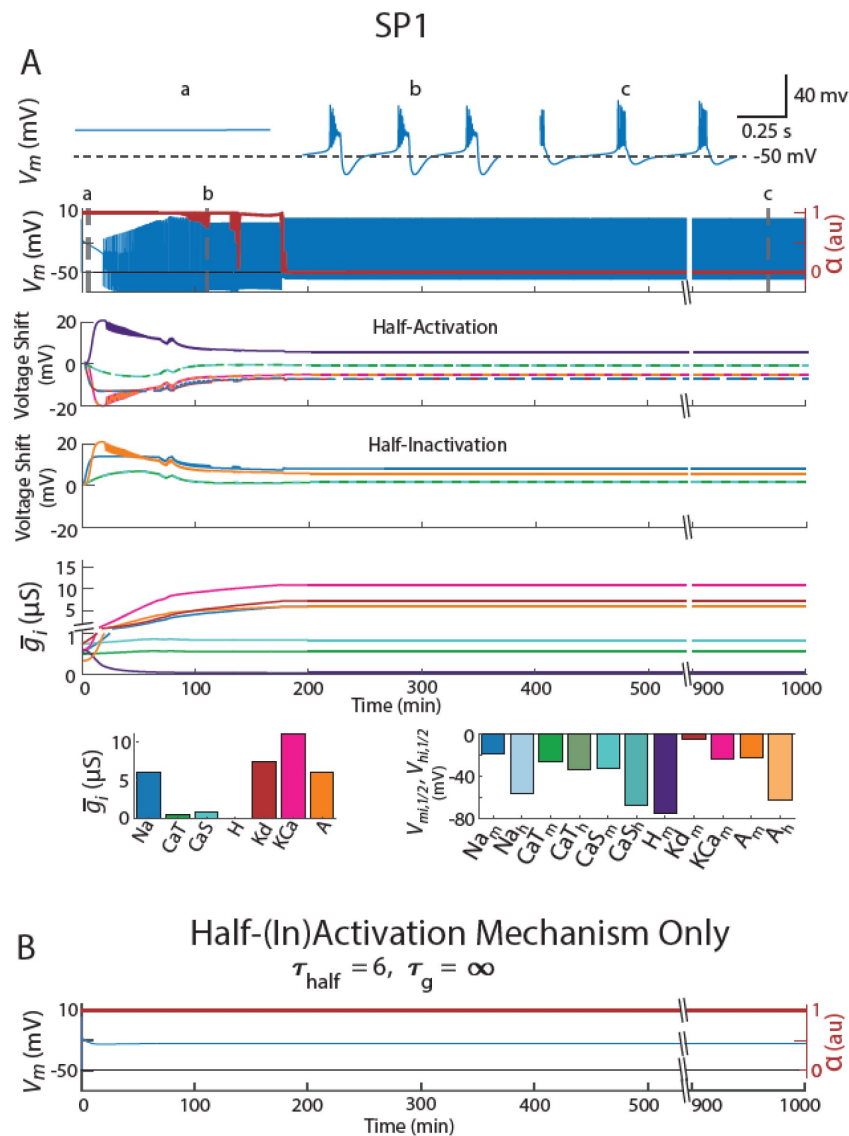
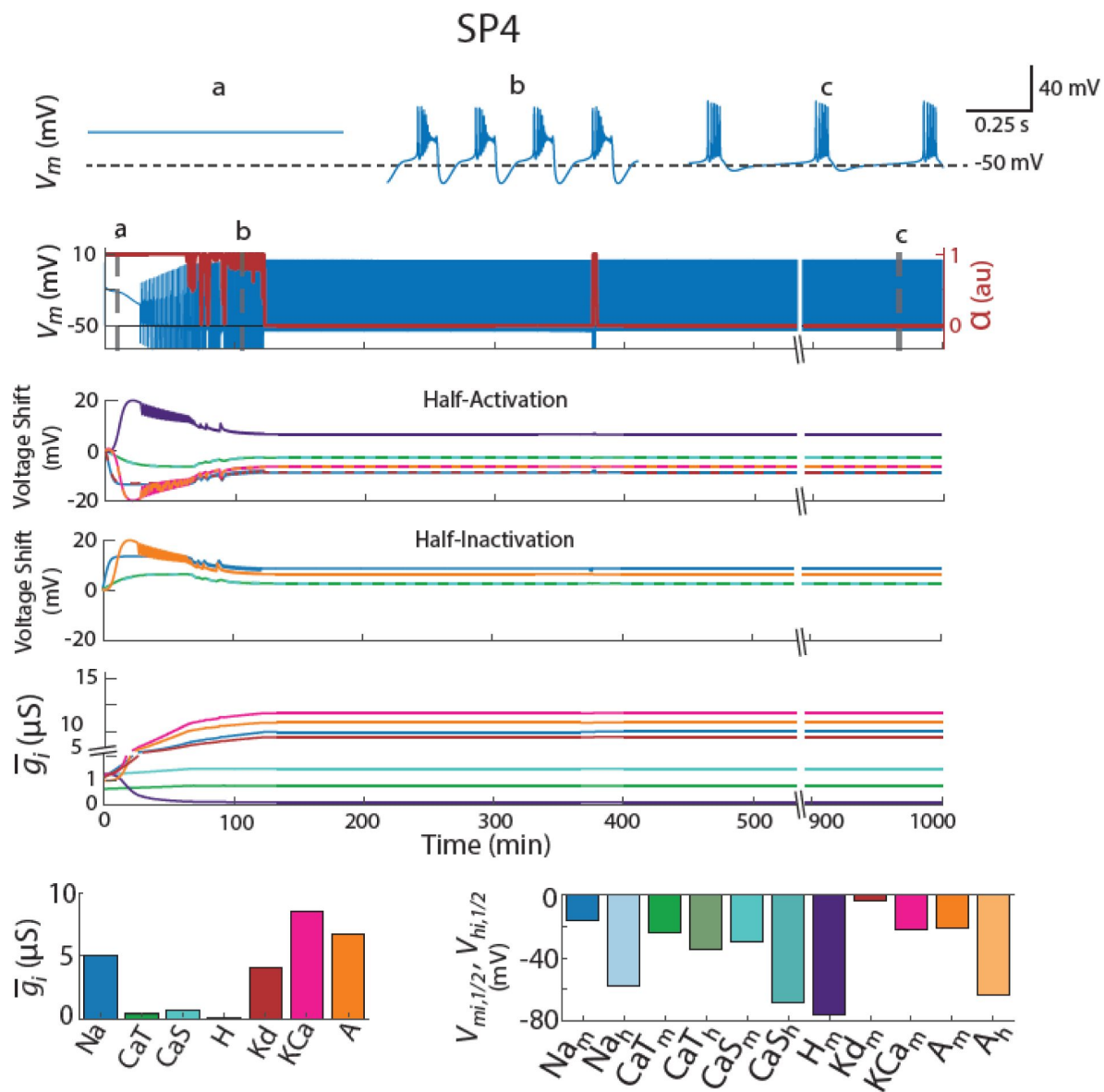


Figure 1

Activity-Dependent Regulation Mechanism ($\tau_{half} = 6$ s and $\tau_g = 600$ s) was used to assemble a burster that meets the Ca^{2+} targets from 'random' starting parameters (i.e. SP).

Both this set of starting parameters and its resultant model are called SP1. A – Row 1: blow-ups of specific points a, b, and c in the voltage trace in Row 2. Row 2: The neuron's electrical activity as its channel density and (in)activations curves were being adjusted. α is a measure of how close the model is to satisfying Ca^{2+} targets (see Methods). It took values between 0 and 1, with 0 meaning the targets were satisfied. Row 3: Plots the adjustments made to the half-activations as the model attempted to satisfy the calcium targets. They are color-coded to match the intrinsic currents shown in the left plot of Row 5. Row 4: Same as Row 3, except for (in)activation curves. Note that not all currents in this model have inactivation curves. Row 5: Same as Row 3, except for maximal conductances (i.e. channel density). Row 6: The final levels of maximal conductances are shown in the left plot. The final half-activation of an intrinsic current, $V_{m_{1/2}}$, was the sum of the initially posited level of the that current's half-activation $V_{m_{1/2}}^0$ and the shifts the model made to the activation curve, $V_{m_{1/2}}^a$ (shown in Row 3). The final half-inactivation of an intrinsic current, $V_{m_{1/2}}$, was the sum of the initially posited level of the that current's half-inactivation $V_{m_{1/2}}^0$ and the shifts the model made to the inactivation curve, $V_{m_{1/2}}^i$ (shown in Row 4). [The process is illustrated in **Figure 1 (supplement 1)** for a different set of starting parameters (i.e. initial half-(in)activation shifts and maximal conductance levels).] B – This displays the neuron's electrical activity if the model was restricted to varying only half-(in)activations ($\tau_{half} = 6$ s), with maximal conductances fixed ($\tau_g = \infty$ s). The same starting parameters as in Panel A were used for this simulation.



Briefly, α measures whether the model has successfully tuned the neuron's ion channel densities and half-(in)activations to create an activity pattern that matches the preset sensor targets; values near zero indicate successful tuning (more in Methods). Even after the model assembles a channel repertoire that satisfies the targets, brief excursions of α away from zero can occur. These excursions exist because the neuron's electrical activity is quasistable—meaning it may lose/add a spike or experience a slight drop in slow wave amplitude, temporarily causing the sensors to be unsatisfied. When this happens, the model quickly makes minor adjustments to the channel densities and half-(in)activations to restore alignment with the targets (**Figure 1—Supplement** [4](#), Row 2, between minutes 300 and 400). These adjustments are minimal (**Figure 1** [4](#), Rows 3–5).

The third and fourth traces in **Figure 1A** [4](#) show how the model altered half-activation and half-inactivation values to meet the Ca^{2+} target. Very early on there were large changes in the activation curves, which then slowly moved to a steady-state value. The fifth trace shows the evolution of maximal conductances. These started to change early on but did so slowly. As the maximal conductances slowly changed, the half-activation curves followed suit, adapting to the new repertoire of ion channels to close the distance to the Ca^{2+} target. **Figure 1 Supplement** [4](#) shows the assembly of a burster from a different set of Starting Parameters (maximal conductances and half-(in) activations (SP4)), with qualitatively similar results to those obtained with SP1 but quantitatively different final parameters.

The results shown in **Figure 1A** [4](#) require activity-dependent regulation of the maximal conductances. When activity-dependent regulation of the maximal conductances is turned off, the model failed to assemble SP1 into a burster (**Figure 1B** [4](#)). This was seen in the other 19 Starting Parameters (SP2–SP20), as well.

In general, when the model was initialized with different Starting Parameters, it produced bursters with different final intrinsic properties. Twenty degenerate bursters (SP1–SP20), conforming to standards specified in Methods, were selected from over 100 bursters produced with different Starting Parameters. **Figure 2A** [4](#) shows the activity characteristics of these 20 bursters (Period, Burst Duration, Spike Height, Maximum Hyperpolarization and Slow Wave Amplitude). The left panel of **Figure 2B** [4](#) shows the range of conductances in this group. The right panel illustrates the variation in half- (in)activation curves. The asterisks indicate the baseline half-(in)activation values; all neuron models were assembled from starting parameters that lay within ± 0.5 mV of these baseline values (see Methods for details).

Half-(in)activation Mechanism Timescale Impacts Activity Pattern

In the previous section, we studied the types of bursters that arose from slowly changing the density of the channel repertoire and quickly altering the half- (in)activations of the resulting repertoire. We next changed the timescale over which we altered half-(in)activations and assessed how the burst characteristics of the population changed (the timescale of channel density alterations remains the same). **Figure 3A** [4](#) shows that alterations in the timescale of half-(in)activation alterations significantly impacted the period, interburst interval, spike height, and maximum hyperpolarization of the entire bursting population.

The timescale of half-(in)activation alterations also impacted some intrinsic properties of the population. **Figure 3B** [4](#) plots the burster assembled with the timescale of half-(in)activation alterations at two extremes: fast and infinitely slow (i.e. off). This visualization is known as a currentscape (Alonso & Marder 2019 [4](#)). In both cases, the resulting burster appeared to terminate using the same mechanism: hyperpolarization via the calcium-activated potassium current. This is seen by the relative contribution the calcium-activated potassium current makes at the point of maximum hyperpolarization (see at the dotted line in **Figure 3B** [4](#)). However, the manner in which this current came to dominate the burster termination differs. Note that the burster constructed without half-(in)activation alterations possessed a higher maximal conductance for

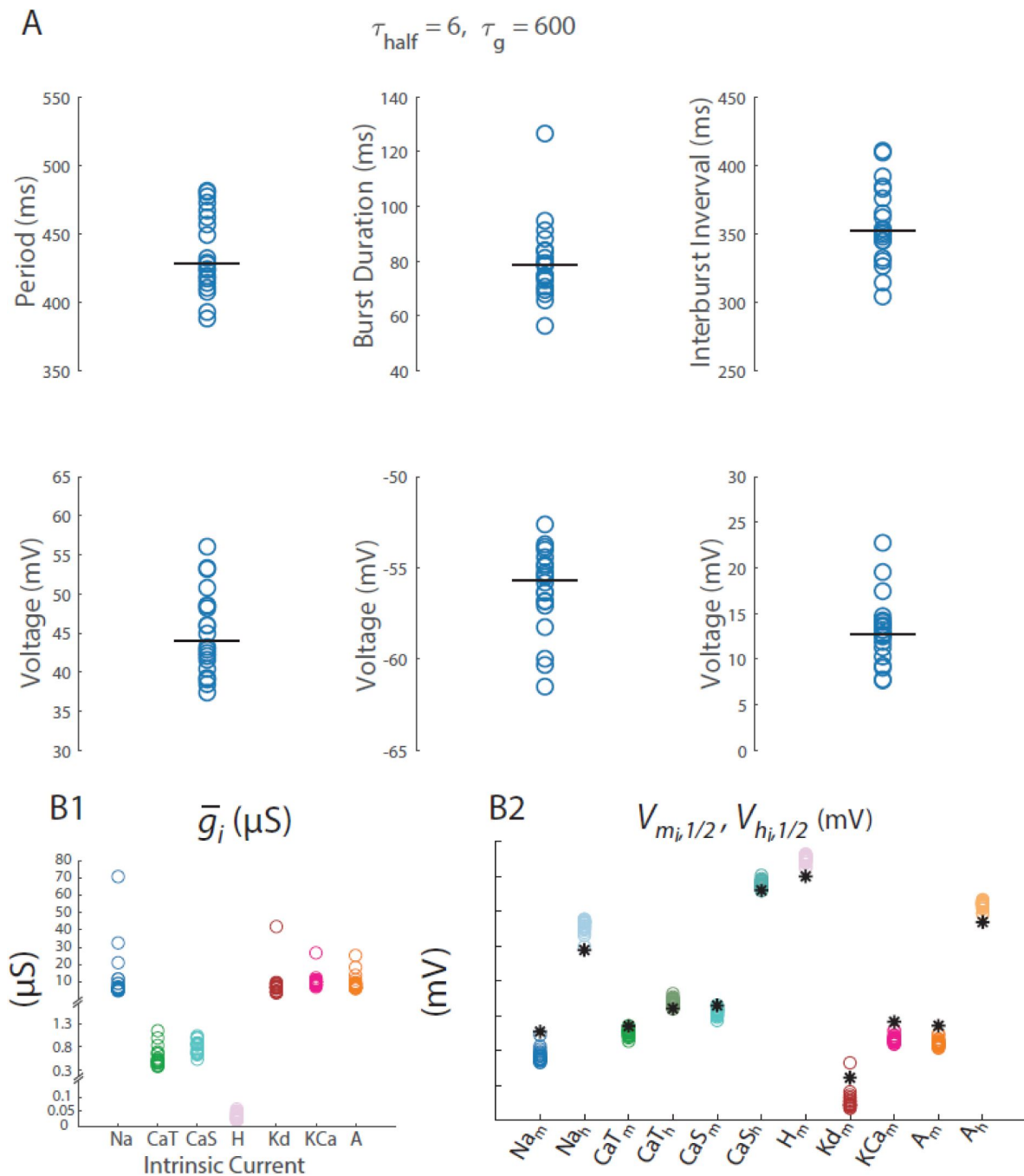


Figure 2

All 20 SP's were analyzed including those illustrated in Figure 1.

A) The top left panel displays the periods for all SP's. Burst Duration, Interburst Interval, Spike Height, Maximum Hyperpolarization, and Slow Wave Amplitude measurements for these SP's are provided in subsequent plots. B) B1 shows maximal conductances, and B2 displays half-(in)activation voltages for each of the 20 SP's. An asterisk on the right indicates the initially specified level of half-(in)activation from which random deviations were made.

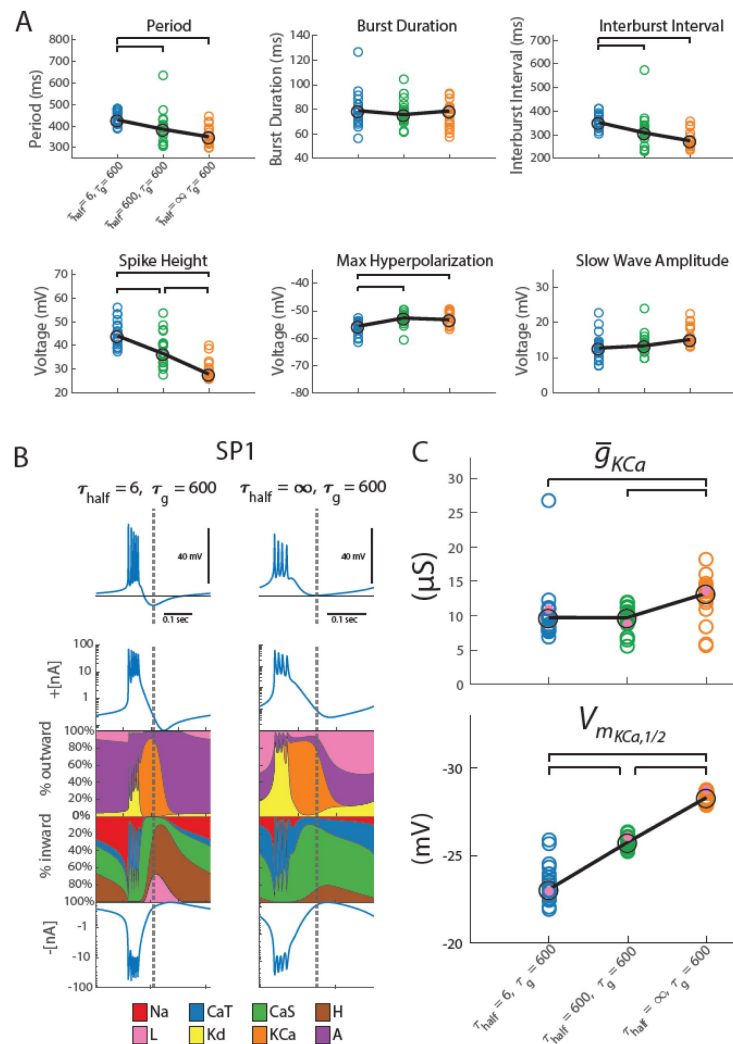


Figure 3

The speed at which the model changed half-(in)activations in response to deviations from Ca^{2+} target impacted the type of bursters the model assembled.

In all simulation for this figure, $\tau_g = 600$. A) These plots extend the measurements from [Figure 2](#) to the same 20 SP's, now with half-(in)activations regulated at a different speed. In blue, $\tau_{half} = 6\text{ s}$; recapitulating results in [Figure 2](#). In green and orange, the SP's were reassessed with slower half-(in)activation adjustments, $\tau_{half} = 600\text{ s}$ and $\tau_{half} = \infty\text{ s}$ (i.e. halted), respectively. Brackets indicate significant differences in medians ($p < .05$). These pairwise differences were assessed using a Kruskal-Wallis test (to assess whether any differences in medians existed between all groups) and post-hoc Dunn tests with Bonferroni corrections to assess which pairs of groups differed significantly in their medians. B) The model was used to stabilize SP1 to the same Ca^{2+} target but using different timescales of half-(in)activation alterations. $\tau_{half} = 6\text{ s}$ is shown on the left and $\tau_{half} = \infty\text{ s}$ (representing the slowest possible response) on the right. Rows 1-4 below each voltage trace show: the total outward current, percentage contribution of each outward intrinsic current to the total outward current, percentage contribution of each inward intrinsic current to the total inward current, and the total inward current. A dashed vertical line across all rows marks the point of maximum hyperpolarization in each activity pattern to guide focused comparison. C) When the model stabilized around a burster, the speed at which the model changed half-(in)activations impacted maximal conductance and half-activation location of the calcium-activated potassium current. Shown here in blue, green, and orange are $\tau_{half} = 6\text{ s}$, $\tau_{half} = 600\text{ s}$, and $\tau_{half} = \infty\text{ s}$ (i.e. halted), respectively. SP1 – SP20 are in the colored circles. Trends are illustrated with a black line, with large circles marking median positions. The mauve dot highlights the levels to which maximal conductance and half-(in)activation of SP1 evolved. The asterisks mark the original position of the KCa half-activation ($V_{mKCa,1/2}^0$). A Kruskal-Wallis test followed by post-hoc Dunn tests (with Bonferroni corrections) were conducted to assess significant ($p < .05$) pairwise differences in medians (brackets).

I_{KCa} than the burster constructed with half-(in)activation alterations (**Figure 3C** [↗](#), Mauve dot). Moreover, the latter burster had more depolarized half-activations (**Figure 3C** [↗](#), Mauve dot). In fact, this observation held true for SP2-SP20 as well (**Figure 3C** [↗](#)).

Potential Mechanism

To explore why the properties of the resulting bursters depend on the timescale of half-(in)activation adjustments, we examined what happens when SP1 is assembled under different half-(in)activation timescales: (1) fast, (2) intermediate (matching the timescale of ion channel density changes), and (3) infinitely slow (i.e., effectively turned off). The effects of these timescales can be seen by comparing the zoomed-in views of the SP1 activity profiles under each condition (**Figure 4** [↗](#)).

When half-(in)activations are fast, the time evolution of α — which tracks how far the activity pattern is from its targets (see Methods)—shows an abrupt jump as it searches for a voltage-dependence configuration that meets calcium targets (**Figure 4A** [↗](#)). As this happens, the channel densities are slightly altered, and this process continues again. Slowing the half-(in)activations reduces these abrupt fluctuations (**Figure 4B** [↗](#)). Making the alterations infinitely slow effectively removes half-(in)activation changes altogether, leaving the system reliant solely on slower alterations in maximal conductances (**Figure 4C** [↗](#)). Because each timescale of half-(in)activation produces a different channel repertoire at each time step, different timescales of half-(in)activation alteration led the model through a different path in the space of activity profiles and intrinsic properties. Ultimately, this resulted in distinct final activity patterns – all of which were consistent with the Ca^{2+} targets.

Half-(in)activation Alterations Contribute to Activity Homeostasis

This model also simulates how the assembled bursters recover their activity following perturbations. In particular, we modeled an increase in extracellular potassium concentration by altering both the potassium reversal potential and the leak reversal potential (see Methods). For these simulations, we used the previously tuned models SP1–SP20. They begin with the homeostatic mechanism engaged but idling because these models already met the prespecified activity targets. However, once extracellular potassium concentration increases, the shifts in reversal potentials cause all 20 models to experience depolarization block. In response, the homeostatic mechanism becomes operative and adjusts the neuron’s maximal conductances and half-(in)activations.

We anticipated that the perturbation would depolarize the membrane. To accommodate this shift, we increased the allowable deviation for the DC calcium sensor, Δ_D (see Methods), from 0.015 to 0.035. **Figure 5A** [↗](#) shows SP1’s activity before perturbation (blue) and after 265 minutes of recovery (red), under three conditions: fast half-(in)activation regulation, intermediate (same timescale as conductance regulation), and no half-(in)activation regulation. The depolarized membrane potential is evident in the more positive maximum hyperpolarizations during perturbation. Notably, although all three cases eventually re-stabilized to activity patterns that satisfied the calcium targets, the resulting burst patterns were different.

SP1 was one of eleven models that successfully recovered under all three homeostatic conditions. Eight models required some form of half-(in)activation modulation to stabilize; in these cases, conductance regulation alone was not sufficient for recovery. Among these eight, two only recovered when half-(in)activation changes were fast, and another two only recovered when they were slow. Evidently, for some models the ability to recover bursting activity depended on both the presence and speed of half-(in)activation modulation.

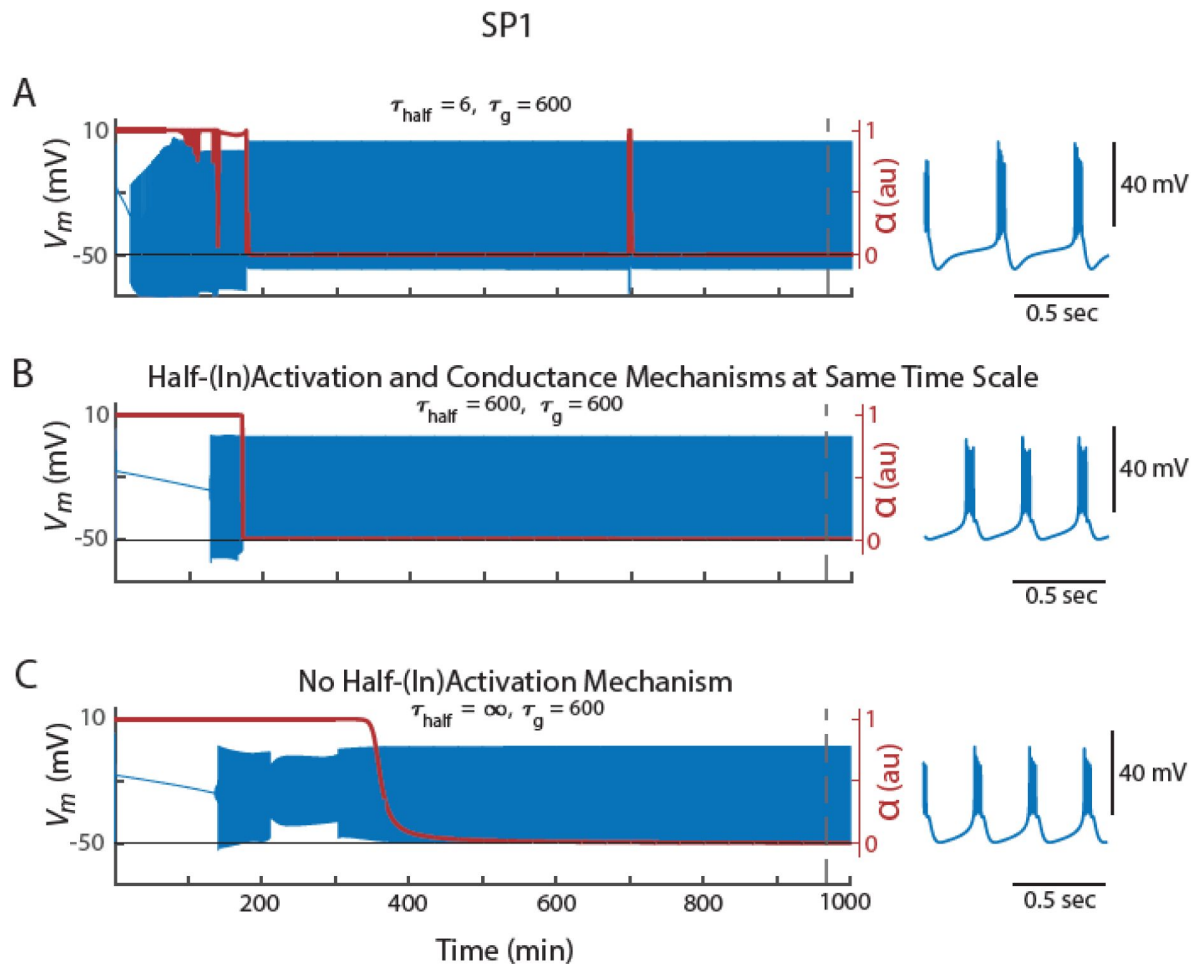


Figure 4

Changes to the timescale of half-(in)activation alterations steers the model through different trajectories in intrinsic parameter space. Each row shows the behavior of Starting Parameter 1 (SP1) as the model adjusts toward a configuration that satisfies the calcium activity target, using different timescales for half-(in)activation regulation.

A – $\tau_{\text{half}} = 6$ s, B – $\tau_{\text{half}} = 600$ s, C – $\tau_{\text{half}} = \infty$ s (i.e. half-(in)activations do not change). In all panels, the speed at which the model changed channel density is fixed in all rows: $\tau_g = 600$ s. Superimposed on these traces is the α parameter, which indicates whether the activity patterns were meeting the Ca^{2+} targets. Blow-ups of the activity pattern when the calcium sensors were satisfied are displayed to the right.

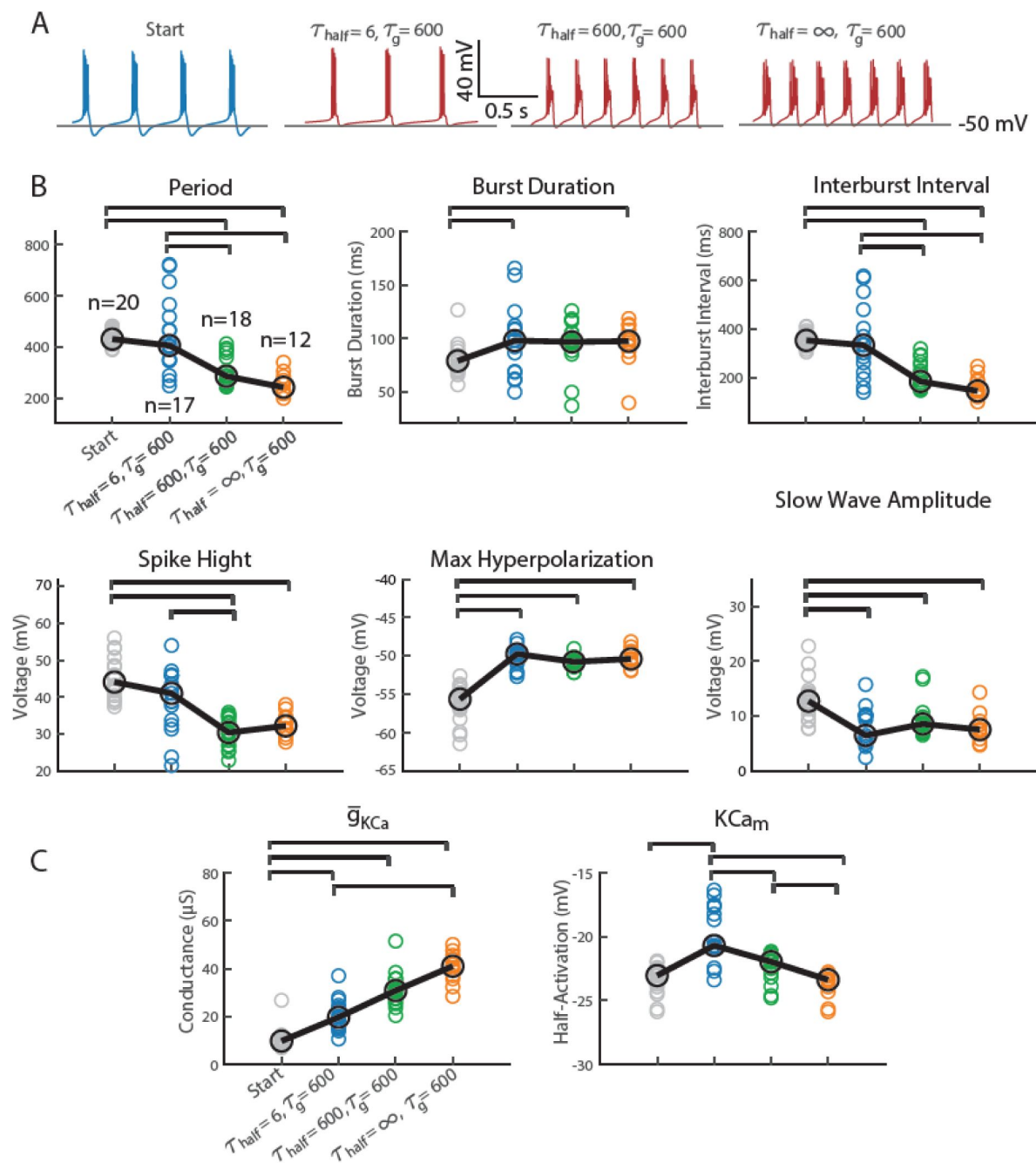


Figure 5

The speed at which the model changed half-(in)activations in response to deviations from Ca^{2+} targets impacted the type of bursters the model assembled following perturbation.

A) Voltage traces from SP1 before (blue) and 265 minutes after (red) a simulated increase in extracellular potassium concentration. Each panel shows recovery under a different timescale for half-(in)activation regulation: fast ($\tau_{half} = 6$ s), intermediate ($\tau_{half} = 600$ s), and infinitely slow (i.e. off, $\tau_{half} = \infty$ s). In all condition, alteration of maximal conductances was held fixed ($\tau_g = 600$ s). B) Summary of activity features for all SP1–SP20 models that recovered to a stable burst pattern. The number of models that successfully recovered varied with the homeostatic mechanism: fast ($n = 17$), intermediate ($n = 18$), and no half-(in)activation modulation ($n = 20$). Gray points indicate pre-perturbation measurements of SP1–20 (from **Figure 3A**). Significant pairwise differences in median values were assessed using a Kruskal–Wallis test followed by post hoc Dunn tests with Bonferroni correction. Significant comparisons ($p < 0.05$) are marked with horizontal brackets. C) The speed of half-(in)activation regulation influenced both the maximal conductance and half-activation voltage of KCa. Data are grouped by as they are in B). Statistical comparisons were performed as in B).

Figures 5B and **5C** quantify the variability in post-recovery activity patterns and intrinsic parameters across different half-(in)activation regulation timescales. Comparing the fast ($\tau_{half} = 6$ s, blue) and intermediate ($\tau_{half} = 600$ s, green) conditions reveals significant differences in burst period, interburst interval, and spike height (**Figure 5B**). Notably, these same features also showed significant variation in **Figure 3A**, where timescale-dependent effects were observed during the initial assembly of bursters. Similarly, the half-activation voltage of the KCa current differs significantly between these two timescales (**Figure 5C**), paralleling the differences observed in **Figure 3C**. In models that successfully recovered from perturbation, the timescale of voltage-dependence modulation influenced both the resulting activity patterns and the intrinsic parameters of the stabilized bursters.

Discussion

Timescale of Channel Voltage-Dependence Alterations

While alterations in channel voltage-dependence are known to modulate a neuron's excitability (Levitan 1988), their role in stabilizing a neuron around an activity target has not been extensively explored. This study explores how modifying the *timescale* of voltage-dependence regulation influences both the initial attainment of a target activity pattern and its maintenance during perturbation. While all models met the activity target regardless of whether voltage-dependence modulation was present, recovery after perturbation depended on both the presence and timescale of this mechanism. Furthermore, in both scenarios, the timescale shaped the stabilized neuron's specific activity characteristics and underlying intrinsic parameters. This suggests a general role for the timescale of half-(in)activation modulation in sculpting stable activity states.

Figure 6 provides a conceptual summary of this idea. The space of possible intrinsic parameter configurations (**Figure 6A**) and electrical activity patterns (**Figure 6B**) each contain regions that satisfy a given activity target—depicted in blue for activity characteristics and green for intrinsic parameters. The perturbations administered in this study simply shift these target-satisfying regions. When channel gating properties are altered quickly in response to deviations from the target activity, the resulting electrical patterns are shown in **Figure 6** as the orange bubble labeled “ $\tau_{half} = 6$ s”. At a slower timescale of alterations, the activity characteristics and model parameters of the electrical patterns shift, as illustrated by the other orange subregions in **Figure 6**. This shift conceptually highlights how changing the timescale of alterations to channel voltage-dependence can move the neuron through different regions within the space of activity characteristics or intrinsic parameters while still maintaining its activity target.

Our simulations show that altering the timescale of the channels' voltage-dependent gating properties impact its activity characteristics. However, what biological processes can implement these alterations? Fast timescale post-translational alterations can be implemented by second messengers that are anchored next to ion channels (Fraser & Scott 1999, Johnson et al 1994) or second messengers that diffuse across the cytosol (Agarwal et al 2016, Zaccolo et al 2006). Phosphorylation by kinases is a commonly studied post-translational modification, however there are other modifications that also occur on these time scales, such as S-nitrosylation (Broillet 1999). On slower timescales, the neuron can modulate the transcription and translation of these auxiliary subunits based on intracellular calcium levels. For instance, Potassium Channel-Interacting Proteins are known to be controlled by CREB (Wu et al 2023).

Biological Relevance

One application for the simulations involving the self-assembly of activity may be to model the initial phases of neural development, when a neuron transitions from having little or no electrical activity to possessing it (Baccaglini & Spitzer 1977). As shown in **Figure 6**, the timescale of

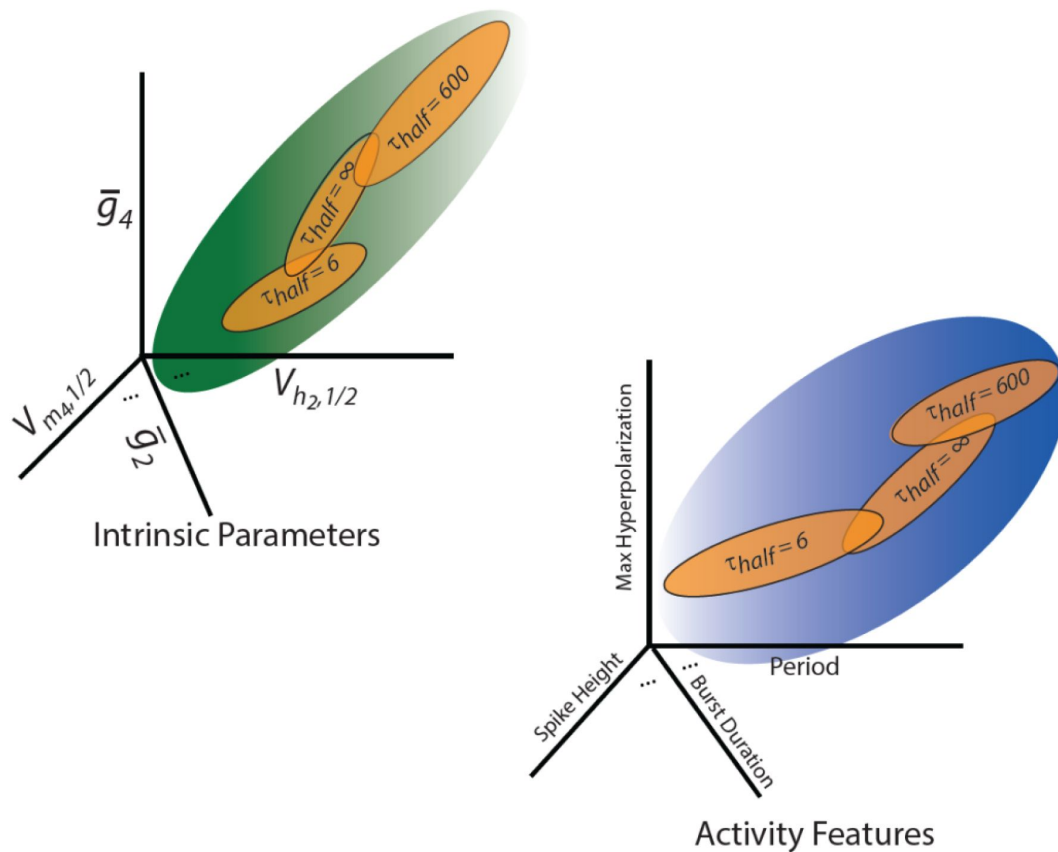


Figure 6

The impact of adjusting the timescale of ion channel (in)activation curve alterations can be illustrated conceptually using the space of activity characteristics (right) and underlying intrinsic parameters (left).

The regions in green and blue are the activity patterns that are consistent with the Ca^{2+} targets in the space of activity characteristics and underlying intrinsic parameters, respectively. As the rate of ion channel half-(in)activation adjustments in response to deviations from Ca^{2+} targets is changed, the regions targeted by the model also shift (shown in orange). Regardless of the timescale, all targeted regions reside within a larger region encompassing all activity profile measurements and intrinsic parameters consistent with the Ca^{2+} targets.

(in)activation curve alterations define a neuron's activity characteristics and intrinsic properties. As such, neurons may actively adjust these timescales to achieve a specific electrical activity aligned with a developmental phase's activity targets. Indeed, developmental phases are marked by changes in ion channel density and voltage-dependence, leading to distinct electrical activity at each stage (Baccaglini & Spitzer 1977 [↗](#), Gao & Ziskind-Conhaim 1998 [↗](#), Goldberg et al 2011 [↗](#), Hunsberger & Mynlieff 2020 [↗](#), McCormick & Prince 1987 [↗](#), Moody & Bosma 2005 [↗](#), O'Leary et al 2014 [↗](#), Picken Bahrey & Moody 2003 [↗](#)).

Additionally, our results show that activity-dependent regulation of channel voltage-dependence can play a critical role in restoring neuronal activity during perturbations (**Figure 5** [↗](#)). Specifically, the presence and timing of half-(in)activation modulation influenced whether the model neuron could successfully return to its target activity pattern. Many model neurons only achieved recovery when a half-(in)activation mechanism was present. Moreover, the speed of this modulation shaped recovery outcomes in nuanced ways: some model neurons reached their targets only when voltage-dependence was adjusted rapidly, while others did so only when these changes occurred slowly. These observations all suggest that impairments in a neuron's ability to modulate the voltage-dependence of its channels may lead to disruptions in activity-dependent homeostasis. This may have implications for conditions such as addiction (Kourrich et al 2015 [↗](#)) and Alzheimer's disease (Styr & Slutsky 2018 [↗](#)), where disruptions in homeostatic processes are thought to contribute to pathogenesis.

In conclusion, our findings suggest that when a neuron begins from a state of no activity and small randomly specified channel densities, changes in channel density are essential for attaining activity. In this context, activity-dependent changes in channel voltage-dependence alone are insufficient to attain bursting (cf **Figure 1B** [↗](#)). Based on this evidence alone, one might conclude that shifts in (in)activation curves have negligible impact, effectively being overshadowed by the larger changes in excitability driven by alterations in channel density. This perspective aligns with a common assumption in systems analysis: that short- and long-timescale processes are sufficiently distinct and can be studied independently without significant interaction. However, our results demonstrate that fast shifts in half-(in)activation constrain the types of activity characteristics and intrinsic properties a neuron ultimately expresses (**Figure 6** [↗](#)). These fast alterations, while subtle, manifest over long timescales required to assemble activity. This study exemplifies how interactions between processes operating on different timescales can influence one another. Such principles are particularly relevant for understanding multiscale interactions in neuroscience, such as the connections between subcellular, cellular, and network-level properties (Bhalla 2014 [↗](#), Marom 2010 [↗](#)).

Methods

Details of the Model and its Implementation

Box 1 [↗](#) presents a scheme for the equations used in the model: a Hodgkin-Huxley type equation with 7 intrinsic currents, Ca^{2+} sensors, and an activity-dependent regulation mechanism.

The Hodgkin-Huxley type neuron consisted of 7 intrinsic currents (**Box 1, Supplement 1** [↗](#), **Equations 1** [↗](#)-**3** [↗](#)) and an intracellular Ca^{2+} concentration calculation (**Box 1, Supplement 1** [↗](#), **Equation 4** [↗](#)). The neuron's capacitance (C) was set to 1 nF. The currents were labeled by the index i and were in order: fast sodium (I_{Na}), transient calcium (I_{CaT}), slow calcium (I_{CaS}), hyperpolarization-activated inward cation current (I_{H}), potassium rectifier (I_{Kd}), calcium-dependent potassium (I_{KCa}), and fast transient potassium (I_{A}). Additionally, there was a leak current (I_{L}). The maximal conductances were given by $\bar{g}_i$. Activation and inactivation dynamics were given by m_i and h_i , respectively. The exponents q_i were given by 3,3,3,1,4,4,3, respectively. The equilibrium/reversal potentials of the sodium current, hyperpolarization activated cation

current, potassium currents, and leak current were +30 mV, -20 mV, -80 mV and -50 mV respectively. The calcium equilibrium potential was computed via the Nernst Equation (Box 1, Supplement 1 [Equation 5](#)) using the computed internal Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) and an external Ca^{2+} concentration of 3 mM (temperature was 10 °C).

We modeled perturbations in neuronal activity by a 2.5-fold increase in extracellular potassium concentration. Using the Nernst equation, this shift corresponds to a change in the potassium reversal potential from -80 mV to -55 mV. We assume that leak current in our model includes a potassium component, so that the perturbation also impacts the leak reversal potential. Additionally, assuming that the remaining leak current comes from sodium channels, we can compute how to perturb the leak reversal potential. The leak maximal conductance for our model is fixed at .01 μS . The new leak reversal potential was calculated using standard computation involving the conductance-weighted average of potassium and sodium reversal potentials, yielding a valued of approximately -32 mV.

The activation curves and inactivation curves were given by $m_{i,\infty}$ and $h_{i,\infty}$. Their associated time constants were τ_{m_i} and τ_{h_i} , respectively. The (in)activation curves and their associated time constants were functions of the membrane voltage, V (see tables in Box 1, Supplement 1 [Equation 4](#)). Note that I_{KCa} had an activation curve that depends on internal Ca^{2+} concentration. The internal calcium concentration was computed using I_{CaT} and I_{CaS} (Box 1, Supplement 1 [Equation 4](#)).

There were three Ca^{2+} sensors that responded to different measures associated with fluctuations of $[\text{Ca}^{2+}]_i$ during a burst. They were used to determine how close these measures are to their targets. These measures were the amount of $[\text{Ca}^{2+}]_i$ that entered the neuron: as a result of the slow wave of a burst (S), as a result of the spiking activity of a burst (F), and on average during a burst (D). They were computed using [Equations 6-8](#) (Box 1, Supplement 2 [Equation 12](#)). Each sensor had a corresponding target value - $\bar{S}$, $\bar{F}$, $\bar{D}$ - which represented the target activity pattern.

The model computed moving averages of the mismatch between each sensor and its target, denoted, E_S , E_F , and E_D ([Equations 9-11](#), Box 1, Supplement 2 [Equation 12](#)). These averages were taken over a two-second window ($\tau_S = 2000$ ms), allowing the model to smooth out short-term fluctuations and capture sustained deviations from the target. Ideally, E_S , E_F , and E_D , are zero, however, ongoing membrane fluctuations, such as bursting or spiking still created small variations in these measures. Allowable tolerances are specified in the model as, Δ_S , Δ_F , and Δ_D , respectively. The moving averages of the mismatches between the calcium sensors and their targets are compared against the allowable tolerances and combined in [Equation 12](#) (Box 1, Supplement 2 [Equation 12](#)) to create a “match score”, S_F ([Equation 12](#)).

To compute this match score, we adapted a formulation from [Alonso et al \(2023\)](#), who originally used a root-mean-square (RMS) or L^2 norm to combine the sensor mismatches. In that approach, each error (E_S , E_F , and E_D) is divided by its allowable tolerance (Δ_S , Δ_F , and Δ_D) to produce a normalized error. These normalized errors are then squared, summed, and square-rooted to produce a single scalar score that reflects how well the model matches the target activity pattern.

In our version, we instead used an L^8 norm, which raises each normalized error to the 8th power before summing and taking the 1/8th root. This formulation emphasizes large deviations in any one sensor, making it easier to pinpoint which feature of the activity is limiting convergence. By amplifying outlier mismatches, this approach provided a clearer view of which sensor was driving model mismatch, helping us both interpret failure modes and tune the model’s sensitivity by adjusting the tolerances for individual sensor errors.

Although the L^8 norm emphasizes large deviations more strongly than the L^2 norm, the choice of norm does not fundamentally alter which models can converge—a model that performs well under one norm can also be made to perform well under another by adjusting the allowable

tolerances. The biophysical mechanisms by which neurons detect deviations from target activity and convert them into changes in ion channel properties are still not well understood. Given this uncertainty, and the fact that using different norms ultimately shouldn't affect the convergence of a given model, the use of different norms to combine sensor errors is consistent with the broader basic premise of the model: that intrinsic homeostatic regulation is calcium mediated.

Equations 13 and 14 were used to evaluate whether the match score was sufficient for the model to cease altering maximal conductances and (in)activation curves, indicating that the target had been achieved. In Equation 14 (Box 1, Supplement 2), the “match score” was compared to a preset threshold value the model would accept, ρ . This equation returned 1 if the match score is below ρ , a 0 if the match score is above ρ , and some number in between 0 and 1 if the match score was close to the threshold, ρ . Equation 13 (Box 1, Supplement 2) then effectively computed a 2 second time average of Equation 14. Equation 13 was an activity sensor, α , that was plotted in Figure 1. It took the value zero when all three Ca^{2+} sensors were close to the Ca^{2+} targets but remained close to one otherwise. The value of this activity sensor was used to slow the evolution of maximal conductances and half-(in)activations as the Ca^{2+} sensors reached their targets. It also served as a measure for how well the model satisfied the Ca^{2+} sensors. In this model, $\rho = 0.3$. The “sharpness” of the threshold at $\rho = 0.3$ was set by Δ_α . We set $\Delta_\alpha = 0.01$ indicating that when the match score, S_f was greater than ~ 0.32 , the model considered the calcium targets satisfied.

Using the information regarding how well the Ca^{2+} sensors were satisfied, the model regulated conductance densities and (in)activation curves. The maximal conductances $\bar{g}_i$, and the ion channel voltage-sensitivities, as measured by activation curve shifts, $V_{s_i^m}$, and inactivation curve shifts, $V_{s_i^h}$, from their specified locations ($V_{m_{1/2}^a}$ for activation curves and $V_{h_{1/2}^i}$ for inactivation curves), were altered by the model in response to deviations from the calcium targets (Box 1, Supplement 3, Equations 15-17). The latter were adjusted on a time scale given by τ_{half} and the former by τ_g . The index i ran from 1 through 7 for the maximal conductances (Equation 15) and half- (in)activations (Equation 16). In Equation 17, the index only took on a subset of values: $i = 1, 2, 3, 7$ – because only some intrinsic currents in this model had inactivation (the associated currents being listed above).

Equations 16 and 17 were new equations added to the model. They were built similarly to Equation 15. In Equation 15, the maximal conductances were altered when the calcium sensors didn't match their targets. There are two parts to Equation 15: a part that converts calcium sensor mismatches into changes in maximal conductances and a cubic term. The cubic term prevents unbounded growth in the maximal conductances. We set the constant associated to the cubic term, γ , to 10^{-7} . The other term adjusted the maximal conductance based on fluctuations of calcium sensors from their target levels. The parameters A_i , B_i and C_i reveal how the sensor fluctuations contribute to the changes of each maximal conductance. These values were provided in Liu et al (1998). Importantly, note that this term is multiplied by $\bar{g}_i$, which prevents the maximal conductance from becoming negative and scales the speed of evolution so that smaller values evolve more slowly and larger values evolve more quickly. As such, multiplication by $\bar{g}_i$ effectively induced exponential changes in the maximal conductance, allowing $\bar{g}_i$ to vary over orders of magnitude.

Equations 16 and 17, introduced in this paper, enable the adjustment of the ion-channel voltage dependence. Equations 16 and 17 also each have two components: a component that converts calcium sensor mismatches into changes in half- (in)activations and a cubic term. The cubic term prevents unbounded translations. The cubic term's constant, $\hat{\gamma}$, was set to 10^{-5} . The other term adjusts the half-activations (Equation 16) and half-inactivation (Equation 17) based on deviation of calcium sensors from their target levels.

Finally, we discuss how the coefficients A_i , B_i and C_i in Equation 15, and the corresponding parameters in Equation 16 and 17 were chosen. The same coefficients used to adjust maximal conductances were used for the inactivation curve shifts, $\hat{A}_i$, $\hat{B}_i$, and $\hat{C}_i$. The coefficients of the activation curve shift are their negatives, $\hat{A}_i$, $\hat{B}_i$, and $\hat{C}_i$. To understand this choice, consider a scenario where the neuron is bursting more rapidly than desired. In that case, then $F > \bar{F}$, $S > \bar{S}$, and $D > \bar{D}$. To reduce the excitability of the neuron, we made the depolarizing current, for example, I_{Na} , harder to activate, by shifting the activation curve of I_{Na} to a more depolarizing potential. This implies $\frac{dV_m}{dt} > 0$. Therefore, the coefficients $\hat{A}_i$, $\hat{B}_i$, and $\hat{C}_i$ must be non-positive. The coefficients for I_{Na} in Liu et al (1998) were given as $A_1 = 1$, $B_1 = 0$ and $C_1 = 0$ and were chosen so that $\frac{d\hat{B}_1}{dt} < 0$, thereby decreasing the maximal conductance of sodium in the same scenario. So, we flipped the signs to give $\hat{A}_1 = -1$, $\hat{B}_1 = 0$ and $\hat{C}_1 = 0$. In this way, only the F Ca^{2+} sensor contributed to the modulation of I_{Na} , but the activation curves were now adjusted to promote homeostasis. Furthermore, the model makes it harder to de-inactivate I_{Na} by shifting its inactivation curve to more hyperpolarized values ($\frac{dV_h}{dt} < 0$). In the scenario in which the activity sensors are greater than their activity targets, this corresponds to setting $\hat{A}_1 = 1$, $\hat{B}_1 = 0$, and $\hat{C}_1 = 0$.

A similar logic can be used to understand how the activation curves of the outward currents were adjusted. In the scenario described above, the neuron was made less excitable by increasing the amount of an outward current, for example I_A . This was done by increasing its maximal conductance or moving its activation curve to more hyperpolarized values. Liu et al (1998) assigned the coefficients $A_7 = 0$, $B_7 = -1$ and $C_7 = -1$ to adjust the maximal conductance of I_A . In this scenario where all sensors are above their targets, this implies $\frac{d\hat{B}_7}{dt} > 0$. Following the prescription we outlined above, flipping the signs gives $\hat{A}_7 = 0$, $\hat{B}_7 = 1$ and $\hat{C}_7 = 1$. This, in turn, implied $\frac{dV_m}{dt} < 0$ – creating hyperpolarizing shifts in I_A 's activation curve when the activity is greater than its target, as we expect. In a similar spirit, we assign the coefficients $\hat{A}_7 = 0$, $\hat{B}_7 = -1$, and $\hat{C}_7 = -1$, so that I_A 's inactivation curve shifts to more depolarizing potentials ($\frac{dV_h}{dt} > 0$) to make it easier to de-inactivate.

Note, the coefficients in Liu et al (1998) don't perfectly correspond with this reasoning. In Liu et al (1998), some coefficients were assigned values to ensure convergence of the model. Still, we followed the prescription outlined above (Box 1, Supplement 3, Table 1).

Starting Parameters

The model has 40 ordinary differential equations: 13 equations describe the neuron's electrical activity, 9 equations describe calcium sensor components, and 18 equations alter the maximal conductances and half-(in)activations (Box 1). This means we needed to specify 40 starting values for the model. We defined starting parameters (SPs), as the bursters the model assembles from a particular set of randomly chosen starting maximal conductances and half-(in)activations. The maximal conductances were randomly selected between 0.3 nS and 0.9 nS and half-(in)activation shifts are randomly offset between -0.5 mV and +0.5 mV from their specified half-(in)activation values ($V_{m1/2}$ and $V_{h1/2}$ in Box 1, Supplement 1, Table 2). Other starting values were $V = -50$ mV and the gating variables of activation and inactivation were randomly selected between 0.2 and 0.3. The starting value of intracellular calcium concentration was set as $[Ca^{2+}]_i = 0.4$ μ M. Equations 9-11 (Sensor Errors) and Equation 13 (Activity sensor, α) were initialized with the value 1. H_X (Equation 7) and M_X (Equation 6) were initialized to 0 and 1, respectively.

The random initialization of gating variables was not expected to significantly impact the formation of an activity profile that satisfied the activity target. Bursters may exhibit bistability if they are initialized with different gating variables. However, this is for a fixed set of maximal conductances and half-(in)activations. Any bistability in the initially specified profile was likely lost as the model altered the maximal conductances and half-(in)activations.

The model was integrated using the Tsitouras 5 implementation of Runge-Kutta 4(5) in Julia's DifferentialEquations.jl package (Tsitouras 2011 [↗](#)). The simulation was saved every 0.5 milliseconds regardless of step size (step size of the integrator was adaptive and left unspecified). Maximum number of iterations was set to 10^{10} and relative tolerance was set to 0.0001.

Burster Measurements

We defined regular bursters as activity patterns that exhibited groups of spikes separated by regular quiescent gaps in activity. To identify bursters, we measured the following activity characteristics: period, burst duration, interburst interval, spike height, maximal hyperpolarization, and slow wave amplitude. Peaks were identified as local maxima in the activity pattern. We used the 'findpeaks' function in MATLAB on the last 90 secs of activity to identify local maxima in the trace, setting the minimum 'prominence' at 2. The latter parameter rejected certain local maxima from counting as peaks. This parameter is one we arrived at by trial and error.

Figures 7-B1 [↗](#) and **7-B2** [↗](#) show red arrows where this function identified peaks. Note in **Figure 7-B2** [↗](#), panels A-C have local maxima that are not counted as peaks (there is one local maxima following the sequence of peaks in the burst). The choice of 'prominence' in the 'findpeaks' function was intentionally set to exclude these. To find troughs, we took the negative of the activity profile and found local maxima using the 'findpeaks' function. For this, the same 'prominence' parameter was used over the same time window. The arrows in **Figure 7C** [↗](#) show an example of what was identified by the algorithm as a peak in this case. Troughs were defined as the negative of the identified points. The red arrow in **Figure 7C** [↗](#) shows the maximum hyperpolarization of a cycle obtained using the method described below. The green arrows are the troughs associated with each spike in the burst (**Figure 7C** [↗](#)).

Maximum hyperpolarization was found as follows. First all troughs were identified in the manner described above. Then, starting at -85 mV, we checked whether there were any troughs below this value. If there were none, we incremented by 1 mV to -84 mV and checked again if there were any troughs below this value. This process was repeated until troughs were detected. The troughs identified this way demarcated the ends of a cycle in the activity pattern. They were averaged to obtain the maximum hyperpolarization measurement for the activity pattern.

The time interval between successive maximum hyperpolarizations in the activity was used to measure the cycle period (**Figure 7D** [↗](#)). We averaged these cycle period measurements to give a measurement for the activity pattern's period. To obtain the burst duration, we segmented the activity pattern into cycles using the time of maximum hyperpolarization. The burst duration of a cycle was measured as the time between the first and last spike in the cycle (**Figure 7C** [↗](#)). The interburst interval of the cycle was computed by subtracting the cycle's burst duration from the cycle period.

To measure the slow wave amplitude, we identified the troughs of each cycle. The slow wave amplitude of a cycle is the difference between the maximum hyperpolarization (**Figure 7D** [↗](#), red arrow) during a cycle and the average of the membrane potential at the troughs associated with each spike in a burst (**Figure 7D** [↗](#), green arrows). The slow wave amplitudes across all cycles were averaged to obtain a measurement for the activity pattern's slow wave amplitude.

The spike height was obtained by finding the peaks of the membrane potential during each cycle. A measure for the cycle's spike height was obtained by averaging over all peaks, subtracting the cycle's maximum hyperpolarization, then subtracting the cycle's slow wave amplitude. This value was averaged over all cycles to obtain a measure of the activity pattern's spike height.

We distinguished regular bursting from tonic spiking or irregular bursting using the following procedure. We classified an activity pattern as a regular burster when: 1) there was more than 1 spike per cycle, 2) the maximum hyperpolarization measurements for all cycles were within 3 mV of one another, and 3) the spikes per cycle varied by no more than 1 spike. The first condition

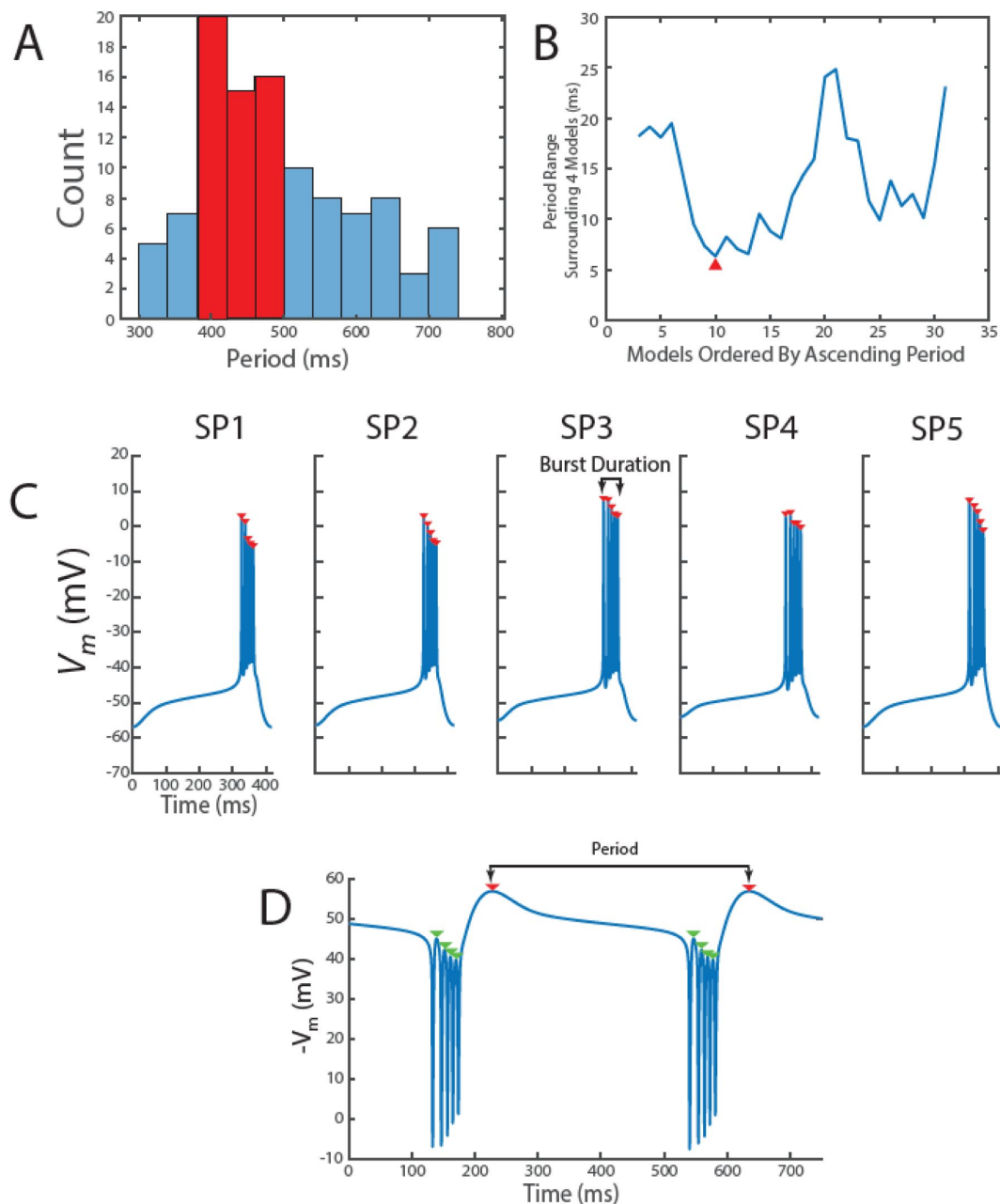


Figure 7

We constructed the Group of 20 Bursters by selecting a putative representative of the entire population and then identifying bursters with similar activity patterns.

A) - 111 bursters were narrowed to 80 bursters by excluding certain characteristics (see Methods). These models had a period distribution shown in A. Highlighted in red are the models chosen for further selection. B) This plot illustrates how closely the periods of the models chosen in A align with their two neighbors after they were ordered from smallest to largest. The first model, SP1, was chosen as the neuron with neighbors that also have close periods (red arrow). C) - To assemble the Group of 20, we symmetrically expanded the inter-instance period range around SP1's period by 20%. SP2-SP5's wave forms are shown. Depolarizing excursions (spikes) were detected using MATLAB's findpeaks function with a prominence threshold of 2 (red arrows). D) - Troughs were identified using the findpeaks function on the negative waveform of a cycle period, applying the same prominence threshold. The detected troughs are indicated by red arrows. The example displayed here represents the inverted voltage trace of the burster assembled by the model from SP1. The period was measured as the time difference between successive points of maximum hyperpolarization.

eliminated tonic spiking, the second condition is a crude measure to check that the average voltage wasn't increasing, and the third checked for irregular bursting. In addition, we verified that the cycle-to-cycle measurements exhibited low variability to ensure the appropriateness of averaging across all cycles. The relative standard deviation for all cycle-to-cycle measurements was less than 3% for the assembled bursters.

Activity Target Selection

We needed a set of sensor targets that *consistently* produced regular bursters, regardless of the starting parameters selected. To determine whether a set of calcium targets consistently produced a burster, we monitored the activity sensor α . α approached 0 when the calcium sensors were close to their specified targets. There were small oscillations in α that resulted from calcium fluctuations associated with the neuron's activity. These fluctuations were smoothed out by taking a running average over the 8 previous seconds. When a simulation fell below $\alpha = 0.02$ for the last time and remained there for 100 minutes, we considered the simulation to have “assembled” a burster that satisfied the calcium targets. For simulations involving elevated extracellular potassium concentration, this criterion was adjusted: a model was considered to have satisfied the calcium targets if the moving average of α fell below 0.02 and remained there for at least 5 minutes. This change was made because the perturbation simulations were shorter.

To ensure the activity pattern obtained did not depend on feedback from the activity regulation mechanism, the mechanism was turned off ($\tau_{half} = \infty$, $\tau_g = \infty$) and the simulation run for an additional 9000 secs to ensure $\alpha < .02$ for the duration. This step was taken in the initial generation of bursters (111 in total, described below), but not for the analysis where the time constants of the half-activation alterations were changed from $\tau_{half} = 6$ s.

‘Consistently’, in this context, meant that the model assembled bursters from over 95% of the randomly chosen starting parameters. The remaining simulations did not complete assembly within the given simulation period. For the calcium targets we found ($\bar{S} = 0.03$, $\bar{F} = 0.25$, and $\bar{D} = 0.02$), the model ‘consistently’ produced bursters. To obtain these targets, we tried different combinations of calcium targets until a suitable set of targets consistently produced regular bursters.

Groups of Bursters

Using the obtained calcium targets, we assembled a total of 111 regular bursters from a wide range of randomly initialized starting parameters. To create a representative subset of this population, we selected a Group of 20 bursters through successive rounds of restriction. The goal was to form a set of models that shared similar burst periods but varied in other activity characteristics and intrinsic properties. This allowed us to assess how different bursters responded to changes in the speed of half- (in)activation alterations as the model attempted to reach or maintain its activity target.

We first filtered the 111 models by slow wave amplitude, retaining only those with amplitudes less than 25 mV. This excluded models with unusually large oscillations and preserved activity patterns with waveforms similar to those shown in [Figure 7C](#), reducing the pool to 80 models. The distribution of these bursters' periods are shown in [Figure 7A](#). The peak of this distribution is between 380 and 500 ms. We further narrowed the population by selecting bursters around the peak of this distribution ([Figure 7A](#), highlighted in Red), resulting in 33 bursters (the “selection group”). This range contained about 30% of the distribution.

In the next round of restriction, we obtained a Group of 20. We ordered the bursters from shortest to longest period. For each burster from the 3rd to the 31st position, we examined the two bursters immediately preceding and the two bursters immediately succeeding in this sorted list and computed the range of burst periods across these five bursters ([Figure 7B](#)). This method

provided a rough estimate of the variability in periods among neighboring bursters. The burster exhibiting the smallest inter-instance period variability was selected as the first member of the Group of 20 (**Figure 7B** [↗](#), Red Arrow). This first burster is referred to as SP1 in **Figure 1** [↗](#) (also shown in **Figure 7C** [↗](#)) and has a period of about 416 ms. In the second step, we symmetrically expanded the inter-instance period range around SP1's period by 20%. We randomly selected an additional 20 models from this group to create the Group of 20.

Model Assumptions

This model of channel voltage-dependence alterations is based on certain assumptions. The concept outlined in **Figure 6** [↗](#) is not expected to depend on these choices. However, examining these assumptions reveals additional insights.

The model makes two simplifications on how quickly channel gating properties can be altered. First, the model assumes a single, uniform time constant for modifying all intrinsic currents. In reality, each intrinsic current is likely influenced by a combination of mechanisms. For example, the relative contributions of anchored second messengers and freely diffusing kinases in phosphorylating key residues in L-Type calcium channels are not well understood and may well involve both processes ([Weiss et al 2013](#) [↗](#)). Consequently, the timescale of modulation for I_{CaS} —the current to which L-Type calcium channels putatively contributes—likely represents a weighted average of these mechanisms. Similarly, I_{Kr} is modulated by PIP2 (a metabolite of calcium-mediated second messenger pathways) and a beta subunit ([Li et al 2011](#) [↗](#)). While the presence or absence of PIP2 can change on a fast timescale, the synthesis of the beta subunit occurs over much slower timescales, resulting in mixed temporal dynamics.

These examples illustrate that the balance between fast and slow regulatory mechanisms likely varies among intrinsic currents, complicating the assumption of a single time constant. Second, our framework assumes post-translational modifications are removed as quickly (or slowly) as they are added. This simplification fails to account for processes where rapid calcium increases lead to prolonged downstream effects. For instance, the calcium-mediated second messenger CaMKII undergoes autophosphorylation, enabling a brief calcium signal to induce long-lasting changes in protein activity ([Hudmon & Schulman 2002](#) [↗](#), [Kennedy 1989](#) [↗](#)). This autophosphorylation is known to influence intrinsic excitability of neurons ([Sametsky et al 2009](#) [↗](#)) and may modulate the voltage-dependence of Ca_v2 channels ([Jiang et al 2008](#) [↗](#)), which putatively contribute to I_{CaS} . Accounting for these processes would require a more complex model capable of capturing asymmetries in modification rates.

Another assumption we make is that there are limits on the extent to which (in)activation curves can shift. This limitation is implemented through the cubic term in [Equations 16](#) [↗](#) and [17](#) [↗](#). These constraints reflect the fact that shifts in activation curves are not unlimited. For example, while cAMP can shift the half-activation of I_h , this effect saturates at high cAMP concentrations ([Difrancesco & Tortora 1991](#) [↗](#)). In this study, however, a uniform constraint is applied across all intrinsic currents. In reality, different intrinsic currents likely have unique limits on how much they can shift, requiring distinct functional forms to model their unique restrictions.

Furthermore, we assumed that activity-dependent alterations in channel voltage-dependence are implemented by changing half-(in)activations. We have not, however, considered the (in)activation curve's slope or the gating variable's time constant. In this study, the gating variable's voltage-dependent time constant shift by the same amount as the half-(in)activation. This preserves how quickly the gating variable respond to changes in voltage – regardless of where the activation curve is centered. However, this coupling between the time constant and the activation curve is not necessarily required. For instance, activity-dependent shifts in voltage-dependent time constants have been observed without concomitant shifts in activation curves ([Thoby-Brisson & Simmers 2002](#) [↗](#)). The voltage dependence of the time constant can, in principle, change in other ways as well. Also, we presume that the slopes of the (in)activation curves do not

respond to changes in activity. The slope of (in)activation curves defines the steepness of the voltage dependence for ion channel gating, thereby influencing the voltage sensitivity of the transition between states (e.g., from closed to open or from inactivated to deinactivated). In this model, the slopes of these curves are held constant.

Consequently, the voltage ranges remain fixed regardless of shifts in the centering of the activation or inactivation curves. Nonetheless, with certain calcium-activated potassium currents, the center and slope of the activation curve depends on calcium concentration (Wang & Brenner 2006 [↗](#)). To the extent that calcium entry during activity initiates downstream signaling processes that alter ion channel function, this observation supports the idea that activity could influence the slope of the I_{KCa} activation curve.

In the model, the same activity targets are used to drive changes in both channel density and voltage dependence. This assumes extensive crosstalk between the calcium pathways posited to be responsible for ion channel insertion, deletion, and post-translational modifications. Nonetheless, the extent to which this crosstalk occurs is not known, although, it is known that cells can detect the temporal patterns and specific pathways of calcium entry, leading to specific activity-dependent changes (Bito et al 1997 [↗](#), Fields et al 1997 [↗](#), Gallin & Greenberg 1995 [↗](#)). Therefore, it is likely that activity-induced calcium influxes trigger changes along distinct pathways – with some impacting only channel gating properties, some impacting channel density, and some impacting both.

It is not necessarily true that all activity-dependent changes in the properties of intrinsic currents are directly controlled by calcium influx. For example, neurons coregulate I_A and I_H expression, so that a change in one current is associated with a change in the other (MacLean et al 2005 [↗](#)). Neuromodulatory environment may also play a role in coordinating ion channel levels (Khorkova & Golowasch 2007 [↗](#)). In such cases, one may use this model for the channels that are controlled by activity and augment it by explicitly modelling the remaining mechanisms that are independent of activity.

Box 1

Model Components

- *Neuron Model*
 - Hodgkin-Huxley representation of a single compartment neuron - voltage- and Ca-dependent currents plus leak
 - Basic $[Ca^{2+}]_i$ handling
- *Ca^{2+} Sensors*
 - Three different sensors that reflect $[Ca^{2+}]_i$ associated with slow wave of activity (S), spiking activity (F) and the average during a burst (D).
 - Targets are set for each sensor independently
 - The three different sensor values are compared to their target values and computed into a single score (α) that reflects the degree to which each is satisfied.
- *Homeostatic Mechanisms*
 - α is used to adjust the maximal conductances on a time scale τ_g and shifts in half-(in)activations voltages on a time scale of τ_{half}

Box 1, Supplement 1

$$(1) \quad C \frac{dV}{dt} = - \sum_{i=1}^7 \bar{g}_i m_i^{q_i} h_i (V - E_i) - g_L (V - E_L) + I_{app}$$

$$(2) \quad \frac{dm_i}{dt} = \frac{m_{i,\infty} (V - V_{s_i}^m) - m_i}{\tau_{m_i} (V - V_{s_i}^m)}$$

$$(3) \quad \frac{dh_i}{dt} = \frac{h_{i,\infty} (V - V_{s_i}^h) - h_i}{\tau_{h_i} (V - V_{s_i}^h)}$$

$$(4) \quad \frac{d[Ca^{2+}]_i}{dt} = \frac{1}{20} (-.94 (I_{CaT} + I_{CaS}) - [Ca^{2+}]_i + .05)$$

$$(5) \quad E_{Ca}([Ca^{2+}]_i) = 12.197 * \log \left(\frac{[Ca^{2+}]_i}{3000.0} \right)$$

I_{CaT} and I_{CaS} are the $i = 2$ and $i = 3$ parts of the sum in Equation 1 [↗](#).

Box 1, Supplement 2

$$(6) \quad \frac{dM_X}{dt} = \frac{\bar{M}_X (I_{CaT} + I_{CaS}) - M_X}{\tau_{M_X}}; X = F, S, D$$

$$(7) \quad \frac{dH_X}{dt} = \frac{\bar{H}_X (I_{CaT} + I_{CaS}) - H_X}{\tau_{H_X}}; X = F, S, D$$

$$(8) \quad F = G_F M_F^2 H_F \quad S = G_S M_S^2 H_S \quad D = G_D M_D^2$$

$$(9) \quad \frac{dE_F}{dt} = \frac{(F - \bar{F}) - E_F}{\tau_S}$$

$$(10) \quad \frac{dE_S}{dt} = \frac{(S - \bar{S}) - E_S}{\tau_S}$$

$$(11) \quad \frac{dE_D}{dt} = \frac{(D - \bar{D}) - E_D}{\tau_S}$$

$$(12) \quad S_f = e^{-(\langle E_F / \Delta_F \rangle^8 + \langle E_D / \Delta_D \rangle^8 + \langle E_S / \Delta_S \rangle^8)^{1/8}}$$

$$(13) \quad \tau_\alpha \frac{d\alpha}{dt} = \alpha_\infty(S_f) - \alpha$$

$$(14) \quad \alpha_\infty(S_f) = \frac{1}{1 + e^{\frac{-(S_f + \rho)}{\Delta_\alpha}}}$$

G_F , G_S and G_D are set to 53, 3, and 1, respectively. Also, $\Delta_F = .1$, $\Delta_S = .008$, and $\Delta_D = .015$. During simulations involving increased extracellular potassium concentration Δ_D is increased to .035

Box 1, Supplement 3

$$(15) \quad \frac{\tau_g}{\alpha} \frac{d\bar{g}_i}{dt} = [A_i(\bar{F} - F) + B_i(\bar{S} - S) + C_i(\bar{D} - D)]\bar{g}_i - \gamma \bar{g}_i^3$$

$$(16) \quad \frac{\tau_{half}}{\alpha} \frac{dV_{s_i}^m}{dt} = [\hat{A}_i(\bar{F} - F) + \hat{B}_i(\bar{S} - S) + \hat{C}_i(\bar{D} - D)] - \hat{\gamma} (V_{s_i}^m)^3$$

$$(17) \quad \frac{\tau_{half}}{\alpha} \frac{dV_{s_i}^h}{dt} = [\hat{A}_i(\bar{F} - F) + \hat{B}_i(\bar{S} - S) + \hat{C}_i(\bar{D} - D)] - \hat{\gamma} (V_{s_i}^h)^3$$

Table 1

Activation Curve Exponents & Equilibrium Potentials

Current	Index (<i>i</i>)	q_i	E (mV)
I_{Na}	1	3	30
I_{CaT}	2	3	[set by Nernst Equation – Equation 5]
I_{CaS}	3	3	[set by Nernst Equation – Equation 5]
I_H	4	1	-20
I_{Kd}	5	4	-80
I_{KCa}	6	4	-80
I_A	7	3	-80

Table 2

Normative values of Half-Activation and Half-Inactivation used to generate all starting parameter sets by randomized shifts. Final parameter sets are converted to the normative value minus the shift.

Current	Index (<i>i</i>)	$V_{\hat{m}_{i,1/2}}^0$ (mV)	$V_{\hat{h}_{i,1/2}}^0$ (mV)
I_{Na}	1	-25.5	-48.9
I_{CaT}	2	-27.1	-32.1
I_{CaS}	3	-33	-60
I_H	4	-70	
I_{Kd}	5	-12.3	
I_{KCa}	6	-28.3	
I_A	7	-27.2	-56.9

Current	$m_{i,\infty}$ (mV)	τ_{m_i} (ms)
I_{Na}	$\frac{1}{1 + \exp\left(\frac{V - (V_{\hat{m}_{1,1/2}^0 + V_{s_1^m})}{-5.29}\right)}$	$1.32 - \frac{1.26}{1 + \exp\left(\frac{(V - V_{s_1^m}) + 120}{-25}\right)}$
I_{CaT}	$\frac{1}{1 + \exp\left(\frac{V - (V_{\hat{m}_{2,1/2}^0 + V_{s_2^m})}{-7.2}\right)}$	$21.7 - \frac{21.3}{1 + \exp\left(\frac{(V - V_{s_2^m}) + 68.1}{-20.5}\right)}$
I_{CaS}	$\frac{1}{1 + \exp\left(\frac{V - (V_{\hat{m}_{3,1/2}^0 + V_{s_3^m})}{-8.1}\right)}$	$1.4 + \frac{7}{\exp\left(\frac{(V - V_{s_3^m}) + 27}{10}\right) + \exp\left(\frac{(V - V_{s_3^m}) + 70}{-13}\right)}$
I_H	$\frac{1}{1 + \exp\left(\frac{V - (V_{\hat{m}_{4,1/2}^0 + V_{s_4^m})}{6}\right)}$	$272 - \frac{-1499}{1 + \exp\left(\frac{(V - V_{s_4^m}) + 42.2}{-8.73}\right)}$
I_{Kd}	$\frac{1}{1 + \exp\left(\frac{V - (V_{\hat{m}_{5,1/2}^0 + V_{s_5^m})}{-11.8}\right)}$	$7.2 - \frac{6.4}{1 + \exp\left(\frac{(V - V_{s_5^m}) + 28.3}{-19.2}\right)}$
I_{KCa}	$\frac{1}{1 + \exp\left(\frac{V - (V_{\hat{m}_{6,1/2}^0 + V_{s_6^m})}{-12.6}\right)} \frac{[Ca^{2+}]_i}{[Ca^{2+}]_i + 3}$	$90.3 - \frac{75.1}{1 + \exp\left(\frac{(V - V_{s_6^m}) + 46}{-22.7}\right)}$
I_A	$\frac{1}{1 + \exp\left(\frac{V - (V_{\hat{m}_{7,1/2}^0 + V_{s_7^m})}{-8.7}\right)}$	$11.6 - \frac{10.4}{1 + \exp\left(\frac{(V - V_{s_7^m}) + 32.9}{-15.2}\right)}$

Table 3

Activation Curves and Associated Time Constants

Current	$h_{i,\infty}$ (mV)	τ_{h_i} (ms)
I_{Na}	$\frac{1}{1 + \exp\left(\frac{V - (V_{\tilde{h}_{1,1/2}^0 + V_{s_1^h})}{5.18}\right)}$	$\left(1.5 - \frac{-1}{1 + \exp\left(\frac{(V - V_{s_1^h}) + 34.9}{3.6}\right)}\right) \left(\frac{.67}{1 + \exp\left(\frac{(V - V_{s_1^h}) + 62.9}{-10}\right)}\right)$
I_{CaT}	$\frac{1}{1 + \exp\left(\frac{V - (V_{\tilde{h}_{2,1/2}^0 + V_{s_2^h})}{5.5}\right)}$	$105 - \frac{89.8}{1 + \exp\left(\frac{(V - V_{s_2^h}) + 55.0}{-16.9}\right)}$
I_{CaS}	$\frac{1}{1 + \exp\left(\frac{V - (V_{\tilde{h}_{3,1/2}^0 + V_{s_3^h})}{6.2}\right)}$	$60 + \frac{150}{\exp\left(\frac{(V - V_{s_3^h}) + 55}{9}\right) + \exp\left(\frac{(V - V_{s_3^h}) + 65}{-16}\right)}$
I_H	1	1
I_{Kd}	1	1
I_{KCa}	1	1
I_A	$\frac{1}{1 + \exp\left(\frac{V - (V_{\tilde{h}_{7,1/2}^0 + V_{s_7^h})}{4.9}\right)}$	$38.6 - \frac{29.2}{1 + \exp\left(\frac{(V - V_{s_7^h}) + 38.9}{-26.5}\right)}$

Table 4

Inactivation Curves and Associated Time Constants

Table 1

M_X Parameters and Time Constants

$\bar{M}_X$ is a sigmoid function with centering parameter Z :

$$\bar{M}_X = \frac{1}{1 + \exp (Z + (I_{car} + I_{cas}))}$$

	Z	τ_{M_X}
F	14.8	0.5
S	7.2	50
D	3	500

$$\bar{M}_X = \frac{1}{1 + \exp (Z + (I_{car} + I_{cas}))}$$

Table 2

H_X Parameters and Time Constants

H_X is a sigmoid function with centering parameter Z :

$$H_X = \frac{1}{1 + \exp(-Z - (I_{CaT} + I_{CaS}))}$$

	Z	τ_{H_X}
F	9.8	1.5
S	2.8	60
D	-	-

$$\bar{H}_X = \frac{1}{1 + \exp(-Z - (I_{CaT} + I_{CaS}))}$$

Table 1

Parameters describing Homeostatic Modulation of Intrinsic Current Properties (Li Matrix)

Current	Index (i)	A_i	B_i	C_i	$\hat{A}_i$	$\hat{B}_i$	$\hat{C}_i$	$\hat{\hat{A}}_i$	$\hat{\hat{B}}_i$	$\hat{\hat{C}}_i$
I_{Na}	1	+1	0	0	-1	0	0	+1	0	0
I_{CaT}	2	0	+1	0	0	-1	0	0	+1	0
I_{CaS}	3	0	+1	0	0	-1	0	0	+1	0
I_H	4	+1	-1	0	-1	+1	0	+1	-1	0
I_{Kd}	5	0	-1	-1	0	+1	+1	0	-1	-1
I_{KCa}	6	0	-1	-1	0	+1	+1	0	-1	-1
I_A	7	0	+1	+1	0	-1	-1	0	+1	+1

In this paper, the values $\hat{A}_i$, $\hat{B}_i$, and $\hat{C}_i$ are the negative of A_i , B_i , and C_i , respectively. The values $\hat{\hat{A}}_i$, $\hat{\hat{B}}_i$, and $\hat{\hat{C}}_i$ are equal to A_i , B_i , and C_i , respectively. The manner at which we arrive at these values are outlined in Methods. As outlined in Discussion, these coefficients do not need to take these values.

Data availability

The code and parameters required to reproduce the results in this work are accessible and at Zenodo. Repository: [<https://github.com/YugarshiM/fastTimescaleHomeostasis>] [DOI: 10.5281/zenodo.12585617]

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Reviewer #1 (Public review):

This revision of the computational study by Mondal et al addresses several issues that I raised in the previous round of reviews and, as such, is greatly improved. The manuscript is more readable, its findings are more clearly described, and both the introduction and the discussion section are tighter and more to the point. And thank you for addressing the three timescales of half activation/inactivation parameters. It makes the mechanism clearer.

Some issues remain that I bring up below.

Comment:

I still have a bone to pick with the claim that "activity-dependent changes in channel voltage-dependence alone are insufficient to attain bursting". As I mentioned in my previous comment, this is also the case for the g_{max} values (channel density). If you choose the g_{max} 's to be in a reasonable range, then the statement above is simply cannot be true. And if, in contrast, you choose the activation/inactivation parameters to be unreasonable, then no set of g_{max} 's can produce proper activity. So I remain baffled what exactly is the point that the authors are trying to make.

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Reviewer #2 (Public review):

Summary:

In this study, Mondal and co-authors present the development of a computational model of homeostatic plasticity incorporating activity-dependent regulation of gating properties (activation, inactivation) of ion channels. The authors show that, similar to what has been observed for activity-dependent regulation of ion channel conductances, implementing activity-dependent regulation of voltage sensitivity participates in the achievement of a target phenotype (bursting or spiking). The results however suggest that activity-dependent regulation of voltage sensitivity is not sufficient to allow this and needs to be associated with the regulation of ion channel conductances in order to reliably reach target phenotype. Although the implementation of this biologically relevant phenomenon is undeniably relevant, a few important questions are left unanswered.

Strengths:

- (1) Implementing activity-dependent regulation of gating properties of ion channels is biologically relevant.
- (2) The modeling work appears to be well performed and provides results that are consistent with previous work performed by the same group.

Weaknesses:

- (1) The main question not addressed in the paper is the relative efficiency and/or participation of voltage-dependence regulation compared to channel conductance in achieving the expected pattern of activity. Is voltage-dependence participating to 50% or 10%. Although this is a difficult question to answer (and it might even be difficult to provide a number), it is important to determine whether channel conductance regulation remains the

main parameter allowing the achievement of a precise pattern of activity (or its recovery after perturbation).

(2) Another related question is whether the speed of recovery is significantly modified by implementing voltage-dependence regulation (it seems to be the case looking at Figure 3). More generally, I believe it would be important to give insights into the overall benefit of implementing voltage-dependence regulation, beyond its rather obvious biological relevance.

(3) Along the same line, the conclusion about how voltage-dependence regulation and channel conductance regulation interact to provide the neuron with the expected activity pattern (summarized and illustrated in Figure 6) is rather qualitative. Consistent with my previous comments, one would expect some quantitative answers to this question, rather than an illustration that approximately places a solution in parameter space.

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Reviewer #3 (Public review):

Mondal et al. use computational modeling to investigate how activity-dependent shifts in voltage-dependent (in)activation curves can complement changes in ion channel conductance to support homeostatic plasticity. While it is well established that the voltage-dependent properties of ion channels influence neuronal excitability, their potential role in homeostatic regulation, alongside conductance changes, has remained largely unexplored. The results presented here demonstrate that activity-dependent regulation of voltage dependence can interact with conductance plasticity to enable neurons to attain and maintain target activity patterns, in this case, intrinsic bursting. Notably, the timescale of these voltage-dependent shifts influences the final steady-state configuration of the model, shaping both channel parameters and activity features such as burst period and duration. A major conclusion of the study is that altering this timescale can seamlessly modulate a neuron's intrinsic properties, which the authors suggest may be a mechanism for adaptation to perturbations.

While this conclusion is largely well-supported, additional analyses could help clarify its scope. For instance, the effects of timescale alterations are clearly demonstrated when the model transitions from an initial state that does not meet the target activity pattern to a new stable state. However, Fig. 6 and the accompanying discussion appear to suggest that changing the timescale alone is sufficient to shift neuronal activity more generally. It would be helpful to clarify that this effect primarily applies during periods of adaptation, such as neurodevelopment or in response to perturbations, and not necessarily once the system has reached a stable, steady state. As currently presented, the simulations do not test whether modifying the timescale can influence activity after the model has stabilized. In such conditions, changes in timescale are unlikely to affect network dynamics unless they somehow alter the stability of the solution, which is not shown here. That said, it seems plausible that real neurons experience ongoing small perturbations which, in conjunction with changes in timescale, could allow gradual shifts toward new solutions. This possibility is not discussed but could be a fruitful direction for future work.

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Author response:

The following is the authors' response to the original reviews.

Reviewer #1 (Public review):

Major comments:

(1) The main issue that I have with this study is the lack of exploration of "why" the model produces the results it does. Considering this is a model, it should be possible to find out why the three timescales of half-act/inact parameter modifications lead to different sets of results. Without this, it is simply an exploratory exercise. (The model does this, but we do not know the mechanism.) Perhaps this is enough as an interesting finding, but it remains unconvincing and (clearly) does not have the impact of describing a potential mechanism that could be potentially explored experimentally.

This is now addressed in a new section in Results ("Potential Mechanism"):

"To explore why the properties of the resulting bursters depend on the timescale of half-(in)activation adjustments, we examined what happens when SP1 is assembled under different half-(in)activation timescales: (1) fast, (2) intermediate (matching the timescale of ion channel density changes), and (3) infinitely slow (i.e., effectively turned off). The effects of these timescales can be seen by comparing the zoomed-in views of the SP1 activity profiles under each condition (Figure 4).

When half-(in)activations are fast, the time evolution of — which tracks how far the activity pattern is from its targets (see Methods)—shows an abrupt jump as it searches for a voltage-dependence configuration that meets calcium targets (Figure 4A). As this happens, the channel densities are slightly altered, and this process continues again. Slowing the half-(in)activation alterations reduces these abrupt fluctuations (Figure 4B). Making the alterations infinitely slow effectively removes half-(in)activation changes altogether, leaving the system reliant solely on slower alterations in maximal conductances (Figure 4C). Because each timescale of half-(in)activation produces a different channel repertoire at each time step, different timescales of half-(in)activation alteration led the model through a different path in the space of activity profiles and intrinsic properties. Ultimately, this resulted in distinct final activity patterns – all of which were consistent with the Ca^{2+} targets [22].

(2) A related issue is the use of bootstrapping to do statistics for a family of models, especially when the question is in fact the width of the distribution of output attributes. I don't buy this. One can run enough models to find say N number of models within a tight range (say 2% cycle period) and the same N number within a loose range (say 20%) and compare the statistics within the two groups with the same N.

We appreciate the reviewer's skepticism regarding our statistical approach with the "Group of 5" and "Group of 20." These groups arose from historical aspects of our analysis and this analysis does not directly advance the main point—that changes in the timescale of channel voltage-dependence alterations impact the properties of bursters to which the homeostatic mechanism converges. Therefore, we removed the references to the Group of 5 and focus on how the Group of 20 responds to variations in the timescale of voltage-dependent alterations.

(3) The third issue is that many of the results that are presented (but not the main one) are completely expected. If one starts with gmax values that would never work (say all of them 0), then it doesn't matter how much one moves the act/inact curves one probably won't get the desired activity. Alternately, if one starts with gmax values that are known to work and randomizes the act/inact midpoints, then the expectation would be that it converges to something that works. This is Figure 1 B and C, no surprise. But it should work the other way around too. If one starts with random act/inact curves that would never work and fixes those, then why would one expect any set of gmax values would

produce the desired response? I can easily imagine setting the half-act/inact values to values that never produce any activity with any gmax.

We appreciate this observation and agree that it highlights a limitation of our initial condition sampling. Our claim that the half-(in)activation mechanism is subordinate to the maximal conductance mechanism is not intended as a general statement. Rather, we make this observation only within the specific range of initial conditions we explored. Within this restricted set, we found that the conductance mechanism was sufficient for successful assembly, while the half-(in)activation mechanism alone was not. We have revised the manuscript to limit the claim.

“The results shown in Figure 1A require activity-dependent regulation of the maximal conductances. When activity-dependent regulation of the maximal conductances is turned off, the model failed to assemble SP1 into a burster (Figure 1B). This was seen in the other 19 Starting Parameters (SP2-SP20), as well [22].

(4) A potential response to my previous criticism would be that you put reasonable constraints on gmax's or half-act/inact values or tie the half-act to half-inact. But that is simply arbitrary ad hoc decisions made to make the model work, much like the L8-norm used to amplify some errors. There is absolutely no reason to believe this is tied to the biology of the system.

Here the reviewer highlights that model choices (e.g., constraints on maximal conductance and half-(in)activation, use of the L8 norm) are not necessarily justified by biology. A discussion of the constraints on maximal conductance and half-(in)activation are in the Model Assumptions section at the end of Methods. The Methods also contains a longer discussion of the use of the L8 norm:

“To compute this match score, we adapted a formulation from Alonso et al (2023), who originally used a root-mean-square (RMS) or norm to combine the sensor mismatches. In that approach, each error (, , and) is divided by its allowable tolerance (, , and) to produce a normalized error. These normalized errors are then squared, summed, and square-rooted to produce a single scalar score that reflects how well the model matches the target activity pattern.

In our version, we instead used an norm, which raises each normalized error to the 8th power before summing and taking the 1/8th root. This formulation emphasizes large deviations in any one sensor, making it easier to pinpoint which feature of the activity is limiting convergence. By amplifying outlier mismatches, this approach provided a clearer view of which sensor was driving model mismatch, helping us both interpret failure modes and tune the model's sensitivity by adjusting the tolerances for individual sensor errors.

Although the norm emphasizes large deviations more strongly than the norm, the choice of norm does not fundamentally alter which models can converge—a model that performs well under one norm can also be made to perform well under another by adjusting the allowable tolerances. The biophysical mechanisms by which neurons detect deviations from target activity and convert them into changes in ion channel properties are still not well understood. Given this uncertainty, and the fact that using different norms ultimately shouldn't affect the convergence of a given model, the use of different norms to combine sensor errors is consistent with the broader basic premise of the model: that intrinsic homeostatic regulation is calcium mediated [22].

(5) The discussion of this manuscript is at once too long and not adequate. It goes into excruciating detail about things that are simply not explored in this study, such as phosphorylation mechanisms, justification of model assumptions of how these

alterations occur, or even the biological relevance. (The whole model is an oversimplification - lack of anatomical structure, three calcium sensors, arbitrary assumptions, and how parameter bounds are implemented.) Lengthy justifications for why channel density & half-act/inact of all currents are obeying the same time constant are answering a question that no one asked. It is a simplified model to make an important point. The authors should make these parts concise and to the point. More importantly, the authors should discuss the mechanism through which these differences may arise. Even if it is not clear, they should speculate.

We agree. A long discussion on Model Assumptions and potential biological mechanisms that implement alteration in channel voltage-dependence obscure this. The former is relocated to the Methods section. The latter discussion is shortened. A discussion of a potential mechanism is included in the Results (Figure 4).

(6) There should be some justification or discussion of the arbitrary assumptions made in the model/methods. I understand some of this is to resolve issues that had come up in previous iterations of this approach and in fact the Alonso et al, 2023 paper was mainly to deal with these issues. However, some level of explanation is needed, especially when assumptions are made simply because of the intuition of the modeler rather than the existence of a biological constraint or any other objective measure.

A discussion of Model Assumptions is included in the Methods.

Reviewer #2 (Public review):

Summary:

In this study, Mondal and co-authors present the development of a computational model of homeostatic plasticity incorporating activity-dependent regulation of gating properties (activation, inactivation) of ion channels. The authors show that, similar to what has been observed for activity-dependent regulation of ion channel conductances, implementing activity-dependent regulation of voltage sensitivity participates in the achievement of a target phenotype (bursting or spiking). The results however suggest that activity-dependent regulation of voltage sensitivity is not sufficient to allow this and needs to be associated with the regulation of ion channel conductances in order to reliably reach the target phenotype. Although the implementation of this biologically relevant phenomenon is undeniably relevant, the main conclusions of the paper and the insights brought by this computational work are difficult to grasp.

Strengths:

- (1) Implementing activity-dependent regulation of gating properties of ion channels is biologically relevant.*
- (2) The modeling work appears to be well performed and provides results that are consistent with previous work performed by the same group.*

Weaknesses:

- (1) The writing is rather confusing, and the state of the art explaining the need for the study is unclear.*

We reorganized the manuscript to make its focus clearer.

Introduction: We clarified our explanation of the state-of-the-art. Briefly, prior work on activity-dependent homeostasis has focused on regulating ion channel density. Neurons have also been documented to homeostatically regulate channel voltage-dependence. However, the

consequences of channel voltage-dependence alterations on homeostatic regulation remain underexplored. To study this, we extend a computational model of activity-dependent homeostasis — originally developed to only alter channel density— to alter channel voltage-dependence.

Results: We reorganized this section to underscore the main point: that the timescale of half-(in)activation alterations influences the intrinsic properties and activity patterns targeted by a homeostatic mechanism. Figures 1A and 1B were retained to provide context—Figure 1A illustrates how activity can emerge from random initial conditions, while Figure 1B suggests that in these simulations, modulation of half-(in)activation played a specific limited role. Figure 2 builds on Figure 1A by summarizing how intrinsic properties and activity characteristics vary across a population of 20 bursters. Figure 3 then demonstrates that despite playing this specific limited role, altering the timescale of half-(in)activation in these simulations significantly impacted the intrinsic properties and activity characteristics of the bursters targeted by the homeostatic mechanism. Figure 4 supports this by offering a possible mechanistic explanation. Finally, Figure 5 reinforces the central message by showing how the same population responds to perturbation when the timescale of half-(in)activation alterations is varied—essentially extending the analysis of Figure 3 to a perturbed regime.

Discussion: The Discussion concentrates on more specifically on how the timescale of half-(in)activation alterations shape bursters targeted by the homeostatic mechanism. Extended content on model assumptions is moved to Methods. The discussion of biological pathways that implement channel voltage-dependence is shortened to avoid distracting from the main message.

Methods: Aside from moving model assumptions here, we removed discussion of the “Group of 5” and explained in more detail why we chose the L8 norm.

(2) The main outcomes and conclusions of the study are difficult to grasp. What is predicted or explained by this new version of homeostatic regulation of neuronal activity?

Our message is general: the timescale of half-(in)activation alterations influences the intrinsic properties and activity characteristics of bursters targeted by a homeostatic mechanism. As such, the implications are general. Their value lies in circumscribing a conceptual framework from which experimentalists may devise and test new hypotheses. We do not aim to predict or explain any specific phenomenon in this work. To address this concern the Discussion highlights two potential implications of our findings—one to neuronal development and another to pathologies that may arise from disruptions to homeostatic processes:

“One application for the simulations involving the self-assembly of activity may be to model the initial phases of neural development, when a neuron transitions from having little or no electrical activity to possessing it (Baccaglini & Spitzer 1977). As shown in Figure 6, the timescale of (in)activation curve alterations define a neuron’s activity characteristics and intrinsic properties. As such, neurons may actively adjust these timescales to achieve a specific electrical activity aligned with a developmental phase’s activity targets. Indeed, developmental phases are marked by changes in ion channel density and voltage-dependence, leading to distinct electrical activity at each stage (Baccaglini & Spitzer 1977, Gao & Ziskind-Conhaim 1998, Goldberg et al 2011, Hunsberger & Mynlieff 2020, McCormick & Prince 1987, Moody & Bosma 2005, O’Leary et al 2014, Picken Bahrey & Moody 2003).

Additionally, our results show that activity-dependent regulation of channel voltage-dependence can play a critical role in restoring neuronal activity during perturbations (Figure 5). Specifically, the presence and timing of half-(in)activation modulation influenced whether the model neuron could successfully return to its target activity pattern. Many model neurons only achieved recovery when a half-(in)activation mechanism was present.

Moreover, the speed of this modulation shaped recovery outcomes in nuanced ways: some model neurons reached their targets only when voltage-dependence was adjusted rapidly, while others did so only when these changes occurred slowly. These observations all suggest that impairments in a neuron's ability to modulate the voltage-dependence of its channels may lead to disruptions in activity-dependent homeostasis. This may have implications for conditions such as addiction (Kourrich et al 2015) and Alzheimer's disease (Styr & Slutsky 2018), where disruptions in homeostatic processes are thought to contribute to pathogenesis."

Reviewer #3 (Public review):

Mondal et al. use computational modeling to investigate how activity-dependent shifts in voltage-dependent (in)activation curves can complement activity-dependent changes in ion channel conductance to support homeostatic plasticity. While changes in the voltage-dependent properties of ion channels are known to modulate neuronal excitability, their role as a homeostatic plasticity mechanism interacting with channel conductance has been largely unexplored. The results presented here demonstrate that activity-dependent regulation of voltage-dependent properties can interact with plasticity in channel conductance to allow neurons to attain and maintain target activity patterns, in this case, intrinsic bursting. These results also show that the rate of channel voltage-dependent shifts can influence steady-state parameters reached as the model stabilizes into a stable intrinsic bursting state. That is, the rate of these modifications shapes the range of channel conductances and half-(in)activation parameters as well as activity characteristics such as burst period and duration. A major conclusion of the study is that altering the timescale of channel voltage dependence can seamlessly shift a neuron's activity characteristics, a mechanism that the authors argue may be employed by neurons to adapt to perturbations. While the study's conclusions are mostly well-supported, additional analyses, and simulations are needed.

(1) A main conclusion of this study is that the speed at which (in)activation dynamics change determines the range of possible electrical patterns. The authors propose that neurons may dynamically regulate the timescale of these changes (a) to achieve alterations in electrical activity patterns, for example, to preserve the relative phase of neuronal firing in a rhythmic network, and (b) to adapt to perturbations. The results presented in Figure 4 clearly demonstrate that the timescale of (in)activation modifications impacts the range of activity patterns generated by the model as it transitions from an initial state of no activity to a final steady-state intrinsic burster. This may have important implications for neuronal development, as discussed by the authors.

However, the authors also argue that the model neuron's dynamics - such as period, and burst duration, etc - could be dynamically modified by altering the timescale of (in)activation changes (Figure 6 and related text). The simulations presented here, however, do not test whether modifications in this timescale can shift the model's activity features once it reaches steady state. In fact, it is unlikely that this would be the case since, at steady-state, calcium targets are already satisfied. It is likely, however, as the authors suggest, that the rate at which (in)activation dynamics change may be important for neuronal adaptation to perturbations, such as changes in temperature or extracellular potassium. Yet, the results presented here do not examine how modifying this timescale influences the model's response to perturbations. Adding simulations to characterize how alterations in the rate of (in)activation dynamics affect the model's response to perturbations-such as transiently elevated extracellular potassium (Figure 5) - would strengthen this conclusion.

The reviewer suggests that our core message — namely, that the timescale of half-(in)activation alterations influences the intrinsic properties and activity patterns targeted by a homeostatic mechanism — should also hold during perturbations. We agree that this

extension strengthens the central message and have incorporated it into the subsection of the Results (“Half-(in)activation Alterations Contribute to Activity Homeostasis”) and Figure 5.

(2) Another key argument in this study is that small, coordinated changes in channel (in)activation contribute to shaping neuronal activity patterns, but that, these subtle effects may be obscured when averaging across a population of neurons. This may be the case; however, the results presented don't clearly demonstrate this point. This point would be strengthened by identifying correlations, if they exist, between (in)activation curves, conductance, and the resulting bursting patterns of the models for the simulations presented in Figure 2 and Figure 4, for example. Alternatively, or additionally, relationships between (in)activation curves could be probed by perturbing individual (in)activation curves and quantifying how the other model parameters compensate, which could clearly illustrate this point.

In part of the Discussion, we noted that small, coordinated shifts in half-(in)activation curves could be obscured when averaging across a population of neurons. Our intention was not to present this as a primary result, but to highlight an emergent consequence of the model: that distinct initial maximal conductances may converge to activity targets via different small shifts in half-(in)activation, making such changes difficult to detect at the population level. However, we did not systematically examine correlations between (in)activation parameters, conductances, and activity features, nor how these correlations might vary with the timescale of (in)activation modulation. While this observation is consistent with model behavior, it does not directly advance the study’s main point — that the timescale of half-(in)activation modulation influences the types of bursting patterns that satisfy the activity target. To keep the focus clear, we have removed this remark from the Discussion, though we agree that a more detailed analysis of these correlations may offer a fruitful direction for future work.

Reviewer #1 (Recommendations for the authors):

Minor comments:

(1) Page 5: remove "an" from "achieve a given an activity..."

The sentence containing this error has been removed.

(2) Page 7, bottom of page. Explain what prespecifying means here. This requires a conceptual explanation, even if the equations are given in the methods. Was one working ad hoc model built from which the three sensor values were chosen? What was this model and how was it benchmarked? The sensors are never shown. In any figure, but presumably they have different kinetics. What is meant by "average value"? What was the window of averaging and why?

The intention of this passage was to provide a broad overview of the homeostatic mechanism, with the rationale for using sensor “averages” as homeostatic targets explained in detail in the Methods. We have replaced the word “average” with “target” to maintain this focus.

(3) Page 9: add "the" in "electrical activity of the neuron as [the] model seeks..."

Done

(4) Page 9: say briefly what alpha is before using it. Also, please be consistent in either using the symbol for alpha or spelling it out across the manuscript and the figures.

Done

(5) Page 10: the paragraph "In general, ..." is confusing although it becomes clear later on what this is all about. Please rewrite and expand this to clarify some points. For instance, the word "degenerate" is first used here and it is unclear in what sense these models are degenerate. Then it is unclear why the first 5 models were chosen and then 15 more added. What was the point of doing this? What is the intent? Set this up properly before saying that you just did it. This also would clarify the weird terminology used later on of Group of 20 vs. Group of 5. The 20 and 5 are arbitrary. Say what the purpose is. Finally, is the "mean" at the very end the same 416 ms? If not, what do you mean by "the mean"? In fact, I find these 2% and 20% to be imprecise substitutes of (say) two distinct values of CV which are an order of magnitude different. Is that the intent?

This comment refers to a passage that was removed during revision.

(6) Page 10: this may be clear to you, but it took me a while to understand that in Figure 1C, you took the working model at the end of 1A, fixed the $g_{\max}$ values and randomized just the half-act/inact values to run it. Perhaps rewrite this to clarify?

This comment refers to a figure that was removed during revision.

(7) Page 13: why do channel densities not change much after the perturbation?

This comment refers to a figure that has since been reworked during revision. In particular, we only study what happens during perturbation. This question is interesting and is the subject of ongoing work.

Reviewer #2 (Recommendations for the authors):

The article should be carefully corrected, because the current quality of writing might obscure the interest of the study. Particular attention should be paid to the state-of-the-art section and to the discussion, but even the writing of the results should be carefully reworked. The current state of the article makes it very difficult to understand the motivation behind the study but also what the main result provided by this work is.

The Introduction, Results, and Discussion have been reworked to build on the central premise of the work: the timescale of half-(in)activation alterations influences the intrinsic properties and activity patterns targeted by the neuron's homeostatic mechanism. These changes are detailed in Public Comment #1.

Reviewer #3 (Recommendations for the authors):

The manuscript presents an interesting computational study exploring how activity-dependent regulation of (in)activation dynamics interacts with conductance plasticity to shape neuronal activity patterns. While the study provides valuable insights, some aspects would benefit from clarification, further analyses, and/or additional simulations to strengthen the conclusions. Below, I outline concerns and comments related to specific details of the model and results presentation that were not included in the public review.

(1) The results presented in Figure 5 show that adaptation occurs in both channel conductances and (in)activation dynamics; however, the changes in conductance remain relatively permanent after the model recovers from the transient elevation in extracellular potassium. It therefore seems likely that the model would recover bursting more quickly in response to a subsequent exposure to simulated elevated extracellular potassium since large modifications in the slowly changing conductances would not be required. If this is the case, it could provide a plausible mechanism for adaptation to

*repeated high-potassium exposure, as demonstrated experimentally in *Cancer borealis* by this group (PMID: 36060056).*

This is an astute observation and the subject of our present follow-up investigation.

(2) In the text relating to Figure 5, it is argued that the resulting shifts in (in)activation curves may be conceptualized as alterations in window currents. It would be helpful to illustrate this by plotting and comparing changes in window currents of these channels alongside the changes in their (in)activation curves.

This comment refers to a passage that was removed during revision.

(3) Some discussion of the role these homeostatic mechanisms may play when the neuron is synaptically integrated into a rhythmically active network could be informative. Surely, phasic and tonic inputs to the neuron would alter its conductance and voltage-dependent properties. Therefore, the model's parameters in an intact network could be very different from those in the synaptically isolated case.

This is an excellent point. We agree that synaptic context—particularly tonic and phasic inputs—would likely influence a neuron's conductances and voltage-dependent properties, potentially leading to different homeostatic outcomes than in the isolated case. While our current study focuses on synaptically isolated neurons, the Marder lab has considered how homeostatically stabilized neurons might interact in network settings. For example, O'Leary et al (2014) presents an example network of three such neurons operating under homeostatic regulation. However, systematically exploring this question remains a challenge. We are currently developing ideas to study this in the context of a simplified half-center oscillator model, where network-level dynamics can be more tractably analyzed.

(4) Why are the transitions of alpha typically so abrupt, essentially either 1 or 0? Similarly, what happens in the model when there are transient transitions from what appears to be a steady-state alpha that abruptly shifts from 0 to 1 or 1 to 0? For example, what is occurring in Figure 1A at ~150s and ~180s when alpha jumps between 1 and 0, or in Figure 1B when the model transiently jumps up from 0 to 1 at ~400s and ~830s? In Figure 1A, does the bursting pattern change at all after ~250s, or is it identical to the pattern at c?

This is addressed in the revision (Lines 141 – 150).

(5) Are the final steady-state parameters of the 25 (sic) models consistent with experimental observations?

It is difficult to assess — it is hard to design an experiment to do what the reviewer is suggesting.

(6) Why isn't g_L allowed to change dynamically? This seems like the most straightforward way to allow a neuron to adjust its excitability (aside from tonic synaptic inputs).

Passive currents could, in principle, be subject to homeostatic regulation. However, our study focused on active intrinsic currents. This focus stems from earlier investigations, which showed that active currents are dynamically regulated during homeostasis – for instance Turrigiano et al (1995) and (Desai et al 1999).

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