Journal Club

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Correlated Voltage Dependences of Ion Channels Revealed

Albert W. Hamood¹ and Marie L. Goeritz²

¹Graduate Program in Neuroscience and ²Volen Center for Complex Systems, Brandeis University, Waltham, Massachusetts 02454-9110
Review of Amendola et al.

A growing body of work, primarily using invertebrate systems, has revealed correlations in the expression levels of different ion channels and thus the amplitude of their respective conductances in neurons (Schulz et al., 2007). One such relationship exists between two currents which, at first approximation, seem to have little in common: I_A , a rapidly inactivating, large-amplitude outward current activated by depolarization; and I_H , a slow, low-amplitude inward current activated by hyperpolarization. Previous work, especially in the stomatogastric nervous system of crustaceans, has uncovered positive correlations between these current densities that are actively maintained even after perturbation by RNA injection (MacLean et al., 2003). Because of such dramatic differences in every facet of these channels' function (kinetics, voltage dependence, reversal potential), linking the observed correlations to function has been challenging, especially given that such experiments and modeling work have suggested that coordinated shifts in the maximal conductance of these channels produce only modest shifts in neuronal output (MacLean et al., 2005).

Interpretations aside, these results have highlighted the importance of considering the specific sets of conductances in individual neurons. Significant vari-

ability exists at the population level in I_A and I_H expression, and studies that average data across neurons can fail to reveal underlying relationships. Modeling work also supports this notion, suggesting that whenever multiple currents contribute to a given firing feature, many conductance sets can produce similar output (Prinz et al., 2004). Uncovering functional relationships between highly variable neuronal features thus requires consideration of the specific characteristics of individual neurons.

A recent paper published in *The Journal of Neuroscience* (Amendola et al., 2012) capitalized on this perspective to uncover a novel form of covariation in the properties of I_A and I_H , potentially revealing the functional role of the observed relationship between these channels and suggesting that consideration of maximal conductance is not enough. Amendola et al. (2012) examined the properties of I_A and I_H in dopaminergic neurons of the substantia nigra (SN) in rat midbrain slice preparations. Although these conductances are quite different and in opposition, they converge on the neuronal behavior of postinhibitory rebound firing to tune the cell's response to inhibitory inputs. Because the neurons examined predominantly receive GABAergic synaptic inputs *in vivo*, this property could be an important target for regulation in this cell type.

Using both long, and short synaptic-like hyperpolarizing pulses to induce rebound firing, Amendola et al. (2012) verified that I_A and I_H indeed have opposing and complementary effects on

rebound firing in these neurons. Application of a selective I_A channel blocker reduced the delay to first spike following hyperpolarization, whereas blocking I_H with a different drug increased it. Using voltage clamp, the authors also measured the biophysical properties of these currents, including current amplitudes and voltage dependences. These data revealed significant variability in both properties. Most interestingly, the authors found a surprising and significant positive correlation between the voltage dependences of I_H activation and I_A inactivation, which was independent of their relative conductances (Fig. 1).

Amendola et al. (2012) next set out to uncover the signals responsible for maintaining this correlation, and found calcium and cAMP levels to be important. This makes sense in light of what is known about these channels in this cell type: I_A channels in SN dopaminergic neurons contain a calcium-sensitive subunit (Liss et al., 2001), while I_H channels in these neurons are sensitive to cAMP (Biel et al., 2009), which is in turn regulated by calcium-sensitive adenylyl cyclases (Chan et al., 2007). By manipulating the levels of calcium and cAMP simultaneously, Amendola et al. (2012) were able to shift the measured voltage dependences of both channels in a coordinated fashion. Mimicking the effect of low intracellular calcium, for example, with a calcium chelator combined with a cAMP analog, caused both the half-maximal inactivation of I_A and the half-maximal activation of I_H to shift to more depolarized potentials (Fig. 1). Interestingly, the re-

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Correspondence should be addressed to Albert W. Hamood, Brandeis University, 415 South Street, M.S. 013, Waltham, MA 02454-9110. E-mail: alhamood@brandeis.edu.

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Figure 1. Covariation of voltage dependences tunes rebound firing. **A**, Although voltage-dependent current properties vary across the population of neurons, shown here schematically for the voltage of I_A half-inactivation ($V_{1/2_inact}$) and I_H half-activation ($V_{1/2_act}$), analysis in individual cells reveals a correlation. In neurons with a relatively hyperpolarized voltage of I_A inactivation (low $I_A V_{1/2_inact}$), I_H also activates at more hyperpolarized voltages (low $I_H V_{1/2_act}$), represented by cells with exclusively yellow I_A and light blue I_H channels. In different sets of neurons, the activation/inactivation voltages for both currents are more depolarized, as depicted by the combination of either orange I_A and blue I_H channels for mid-range levels, or red I_A and dark blue I_H channels in cells with very depolarized levels of I_A inactivation and I_H activation. The different number of channels within individual cells in the schematic reflects the fact that the maximal conductance of I_A and I_H were not correlated in the population of tested neurons. **B**, Signaling based on intracellular calcium concentration shifts rebound properties. Reduced intracellular calcium availability, which causes an increase in cAMP in SN neurons, depolarizes the voltage dependences of I_A and I_H (illustrated in the shift from yellow to red, and from light blue to dark blue, respectively) and thus causes an increased response to inhibitory inputs, as shown by a decreased delay to first spike following the release of inhibition.

sulting voltage dependences remained within the range measured in the control population, simply clustering at the most depolarized values with reduced variability.

While these correlative results are intriguing, Amendola et al. (2012) also showed a causal relationship between covariation of I_A and I_H voltage dependences and rebound firing by using dynamic clamp, an electrophysiological technique in which ionic conductances are computationally simulated in living neurons by dynamic current injection. Coordinated shifts produced significant changes in the delay of rebound firing: depolarizing both voltage dependences reduced this delay, whereas hyperpolarizing both increased it. Conversely, uncoordinated shifts, by which the voltage dependence of one current was depolarized and the other hyperpolarized, did not produce a significant change. In contrast, coordinated shifts in the maximal conductances of these two

currents, which maintained the ratio between them and simulated correlated shifts in expression levels, tended to maintain rebound delay properties, recapitulating results seen in invertebrate work.

These last results, in particular, highlight the danger of considering only correlations in maximal conductance when trying to understand the function of coregulation of ion channels. Although I_A and I_H are important for tuning postinhibitory rebound firing, merely shifting their current densities together has little impact. Shifting their voltage dependences, however, may be an efficient mechanism by which neurons can actively adjust their rebound firing to meet functional requirements. Amendola et al. (2012) describe the covariation of maximal conductances as a homeostatic mechanism, and covariation of voltage dependences as essentially nonhomeostatic, yielding instead a tuning effect that increases the neuron's dynamic range.

This latter effect may indeed be the more important physiological outcome, and this potentially efficient mechanism for tuning output may explain the previously described relationship between these currents; this conclusion is unreachable when only overall channel expression level is considered.

While Amendola et al. (2012) describe this tuning ability as nonhomeostatic, such a pattern of regulation can conceivably fit into a larger scheme of homeostatic regulation by which the cell aims to maintain a target level of activity. In isolated cultured neurons of the stomatogastric ganglion, for example, prolonged activity deprivation leads to an upregulation of intrinsic excitability (Turrigiano et al., 1995). In cultured mammalian visual cortical neurons, TTX treatment leads to an increase in the strength of incoming excitatory synapses (Turrigiano et al., 1998). These changes have been considered homeostatic as they respond dynamically to decreased neuronal activity in ways that make future activity more likely. Intracellular calcium has been seen as a likely catalyst for these changes, given its utility as a neuronal activity sensor (Ross, 1989). In this context, the mechanism and signaling pathway uncovered by Amendola et al. (2012) could indeed serve a homeostatic function. By depolarizing the voltage dependences of I_A and I_H in response to drops in intracellular calcium, the cell increases its likelihood of rebound firing, in theory increasing its activity level in response to a signal for low activity. By hyperpolarizing these voltages in response to high levels of intracellular calcium, the opposite effect is achieved. Thus, this fast, coordinated regulation could provide a first line of homeostatic defense, allowing the neuron to rapidly adjust its excitability in response to its recent history of activation, potentially maintaining its firing rate in a maximally functional range.

This intriguing, novel relationship between the voltage dependences of distinct ion channels not only has important consequences for neuronal function, but again demonstrates the importance of measuring multiple electrophysiological properties in the same neuron to understand such relationships. An analysis of these data averaged across the neuronal population would reveal only significant variability, completely obscuring the important discovery of Amendola et al. (2012). Much work is now needed to extend these findings to other systems and

cell types. We suggest that this mechanism might play an important role in activity-dependent homeostasis, an idea that remains to be explored, and that the availability of this tuning mechanism could explain the previously described relationship between the expression of these two channels. While regulation at the level of maximal conductance certainly exists, ion channels themselves are subject to a wide variety of regulatory mechanisms that can play important functional roles. Voltage dependences are one possible target of such regulation, as are other factors such as the kinetics of activation and inactivation. How neurons are able to navigate this complicated landscape of regulatory pathways and produce coherent, functional change relevant for their behavior is a complex puzzle that will continue to be a focus of future work.

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