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Who Let the Spikes Out?

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Are you impulsive, or do you tend to be more deliberate? Have you ever felt the need to be cautious only to be dragged into something by a reckless accomplice? In this issue Hu et. al provide compelling evidence for molecular peer-pressure: two sodium channel (NaCh) isoforms with different demeanors located in the axon initial segment (AIS) – one a bit cautious and the other more impetuous. Neurons are continuously barraged by synaptic input which opens neurotransmitter receptors and induces changes in membrane potential (V_m). Once V_m becomes sufficiently elevated, voltage-gated NaChs open and initiate an action potential (AP), or spike, fulfilling the neuron's role as an information integrator. Previous studies have shown that the AIS, a structure at the juncture between the soma and the axon, is rich in NaChs¹ and initiates APs². Once initiated, spikes propagate in two directions, forward down the axon to cause neurotransmitter release by depolarizing presynaptic terminals³, and backwards through the soma and then on to the dendrites. While the forward-propagating AP transmits information to downstream post-synaptic neurons, the back-propagating AP enables forms of synaptic plasticity^{4,5}. The unique characteristics of the AIS that allow it to both initiate spikes with relative ease and then guarantee subsequent back-propagation have remained elusive.

Here, Hu and colleagues beautifully show, using quantitative immunostaining, electrophysiology (including the novel method of axonal bleb recording developed by the senior author) and computer modeling, that two NaCh sub-types, the high-threshold $Na_v1.2$ and the low-threshold $Na_v1.6$, are asymmetrically distributed in the AIS - precisely localizing these NaChs within the complex topography of the neuron. $Na_v1.2$ is found mainly in the 25 μm of the AIS closest to the soma, and requires substantial depolarization for activation. $Na_v1.6$, on the other hand, is found in more distal portions of the AIS, 25-50 μm from the soma, and is activated by relatively little depolarization⁶. This polarized configuration, low-threshold NaChs in the distal AIS flanked by high-threshold NaChs closer to the soma creates a new blueprint of AIS function which explains many of the unique properties of the AIS, including the faithful generation of back-propagating APs.

In this new model, APs are detonated by $Na_v1.6$ channels because of their low threshold for activation and high channel density⁷. $Na_v1.6$ channels sit in the perfect location to allow their easy initiation of APs – distal to the incoming dendritic excitation and insulated from it by somatic inhibitory neurotransmission and a reserve pool of timid $Na_v1.2$ channels in the proximal AIS. If synaptic depolarization makes it as far as the distal AIS, the trigger-happy $Na_v1.6$ figures the neuron deserves to spike. Once $Na_v1.6$ channels are activated they rapidly depolarize the nearby area, coercing the hesitant $Na_v1.2$ channels in the proximal AIS to open and generate a back-propagating AP. Having a reserve of high-threshold $Na_v1.2$ channels proximal to the soma, the majority of which fail to open in response to the initial synaptic depolarization, provides a source of non-inactivated NaChs ready and waiting to initiate a back-propagating AP. Furthermore because $Na_v1.6$ channels in the

distal AIS have entered an inactive state by the time $\text{Na}_V1.2$ channels open, a second forward-propagating AP is prevented. Although elements of this scheme are not perfectly clear, this mechanism of spike initiation followed by faithful generation of a back-propagating AP is both alluring and exciting.

The initiation of APs in the AIS is not a new concept. In fact the mechanism proposed above draws on years of work from groups dedicated to understanding the specific mechanism of spike generation. Eccles and colleagues first reported over 50 years ago that the AP appears first in the AIS of motoneurons and is followed by a back-propagating somatodendritic AP². As electrophysiological and imaging techniques advanced so did our understanding of spike initiation. Pioneering studies by Colbert & Johnston⁸ used simultaneous recording from the soma and AIS of subicular neurons to demonstrate that during an AP, V_m rises more rapidly in the AIS, which occurs presumably due to the $\text{Na}_V1.6$ localization found by Hu et al. In the soma, Colbert and Johnston showed that the onset of a spike occurs more slowly initially, due to what we now think is $\text{Na}_V1.6$ -mediated depolarization in the distal AIS, and is then followed by a rapid increase in V_m which now appears to be driven by $\text{Na}_V1.2$ activation in the proximal AIS. Colbert & Johnston also show that somatic AP threshold is established by sodium channels $\approx 50 \mu\text{m}$ from the soma, where Hu et. al have localized $\text{Na}_V1.6$. Although these authors did not know the identity of the NaChs subtypes driving APs, they proposed the idea of a 'heminode' beyond the AIS where APs originate – an idea that is conceptually validated by Hu et. al's finding of a high concentration of $\text{Na}_V1.6$ channels in the distal AIS.

Recently, Kole & Stuart⁹ unraveled a long standing mystery – if AIS NaCh density explains spike initiation, then why don't recordings reveal a higher density of NaChs in the AIS than elsewhere in the neuron? Answering this question required a literal deconstruction of the AIS, a structure notorious for its dense cytoskeleton¹⁰. The AIS is rich in the adapter protein ankyrin G which helps cluster both NaChs¹¹ and potassium channels¹². Kole & Stuart demonstrated that disruption of the actin cytoskeleton, and presumably its ability to stabilize ankyrin G, caused a three-fold increase in the sodium current that could be recorded in the AIS of layer V pyramidal neurons⁹. This leads one to the hypothesis that the AIS is designed to be a hospitable home to NaChs, not to patch pipettes. The rigid cytoskeletal scaffolding which keeps NaChs entrenched at the AIS causes the membrane the channels exists in to be so inflexible that they cannot be pulled into the pipette tip for patch recording. These results thus confirmed immunohistological and sodium-imaging findings and reconciled previous electrophysiological findings. Overall these results highlight the high value neurons place on bidirectional spike propagation. They have evolved an anatomical distribution of NaChs at a location distinct from that of incoming synaptic input and developed an extensive cytoskeletal system to ensure its stability.

Hu and colleagues also address a recent controversy in the spike generation field - the possibility that NaCh activation is a cooperative process^{13,14}. When APs are recorded from the soma of layer V cortical neurons, their onset is so rapid that some believe they cannot be described using classic Hodgkin-Huxley models, but can be recreated if NaCh gating is cooperative. According to the cooperative gating model, the statistical probability of any given channel opening in an environment rich with NaChs, such as the AIS, would not only be determined by V_m but also by the open-state of nearby NaChs. However, Hu et. al report that neither partial blockade of voltage-gated NaChs with tetrodotoxin nor decreasing NaCh currents with a low sodium buffer alters the voltage dependence of channel activation. If NaCh activation was cooperative, one would expect that removing a subset of NaChs from the active pool of channels with tetrodotoxin would alter channel activation, while reducing the sodium driving force would not. This result should lay to rest the notion that unique,

cooperative, NaCh gating occurs in the AIS to initiate APs and supports the idea that the rapid onset of APs in the soma results from recording distally from the site of AP initiation.

Is there a new integrated view of spike initiation in pyramidal neurons? Hu and colleagues combined their electrophysiological and immunohistochemical findings with elegant modeling experiments to confirm the roles of $\text{Na}_V1.6$ and $\text{Na}_V1.2$. By altering the relative amounts of $\text{Na}_V1.2$ and $\text{Na}_V1.6$ in their model the authors show that the forward propagating AP threshold is almost completely dependent on the impulsive $\text{Na}_V1.6$, while the threshold for generating a back-propagating somatodendritic AP is controlled by the hesitant $\text{Na}_V1.2$. It will be exciting to see which other unique biophysical parameters of $\text{Na}_V1.2$ and $\text{Na}_V1.6$ will be relevant to additional aspects of spike generation and neuronal excitability. Will the faster recovery from inactivation seen in $\text{Na}_V1.2$ mean that they are more responsible for AP generation during high-frequency firing? Or will the ability of $\text{Na}_V1.6$ to maintain high current amplitude during repeated activation put it in the driver's seat during high-frequency spiking⁶? Will the differential effects of drugs modulating NaCh properties, i.e. phenytoin, carbamazepine, lamotrigine, etc, be better understood now that we know more about the specific ion channels mediating AP generation? With this detailed picture of the spike generation machinery we are much better equipped to answer these and other pressing questions.

As a final note, our understanding of spike generation has truly paralleled our technical advances in electrophysiological and imaging techniques. From Eccles and colleagues intracellular recordings from motoneurons, to our ability to make simultaneous patch clamp recordings from a single neuron at multiple locations, to *in vivo* recording of AP threshold, our knowledge of spike initiation continues to grow. Now techniques like voltage and sodium imaging and bleb recording are rapidly advancing our ability to characterize excitability in specific neuronal sub-structures. The most intriguing question that Hu and colleagues leave unanswered is how is NaCh distribution built and maintained? Which cytoskeletal components, signaling molecules, and NaCh domains are responsible? Does inappropriate trafficking or anchoring of NaChs underlie pathological states? The ion channel trafficking and cytoskeletal interaction which have been so elegantly studied in the synapse now must be understood in the AIS.

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Figure.

A New Blueprint for Action Potential Initiation in the Axon Initial Segment. Excitatory neurotransmission onto the dendrites of a layer V pyramidal cell (green oval) causes depolarization of the post-synaptic V_m . This local depolarization moves electrotonically towards the soma (green arrows), where it can be shunted by inhibitory GABAergic neurotransmission (red oval). However, with sufficient synaptic input, depolarization will spread beyond the soma and into the axon initial segment (inset). Once incoming depolarization (green arrow) reaches the AIS (1) it will first enter an area rich in $\text{Na}_v1.2$ (blue). These channels are the “cautious” high-threshold subtype so depolarization will pass through without rapidly activating the $\text{Na}_v1.2$ channels (green arrows with white dashes). It then enters the yellow region, where it rapidly activates the $\text{Na}_v1.6$ channels (red arrows with red dashes), leading to the initiation of an action potential (labeled 3).

When the wave of depolarization reaches the “trigger-happy” low-threshold $\text{Na}_V1.6$ channels (yellow), however, they will quickly open (2) and initiate an inward sodium current (red arrow). This will rapidly depolarize V_m in the distal AIS, activating other nearby $\text{Na}_V1.6$ channels causing a chain reaction of NaCh opening and initiating a forward-propagating action potential (3). Because $\text{Na}_V1.2$ channels were bypassed by the initial synaptic depolarization they are available for activation, rather than in an inactivated state. When $\text{Na}_V1.6$ channels open they drive $\text{Na}_V1.2$ channel activation (4), inducing a secondary wave of inward sodium currents and initiating a back-propagating action potential (5). Because $\text{Na}_V1.6$ channels will be in their inactive state, $\text{Na}_V1.2$ channel opening will not induce a secondary forward-propagating AP.