Who let the spikes out?

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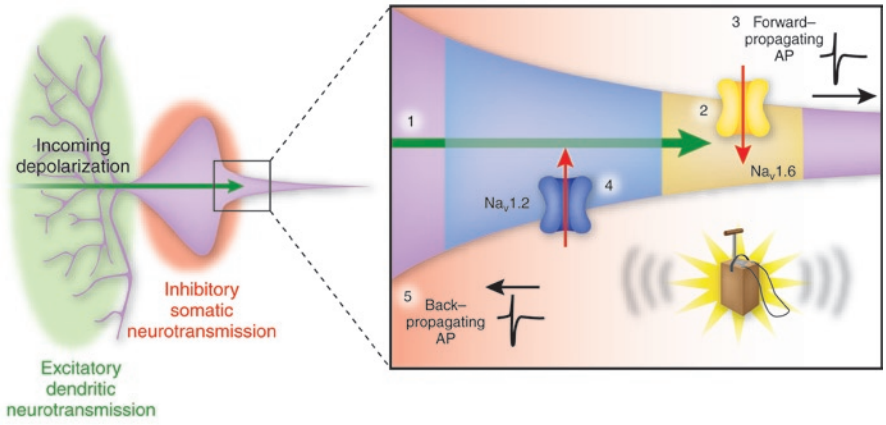
Quantitative immunostaining, electrophysiology and modeling show that two sodium channel isoforms are asymmetrically distributed in the axon initial segment. Their polarized distribution explains many of the unique properties of the axon initial segment, including its ability to both initiate spikes and guarantee subsequent backpropagation.

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.*[1] provide compelling evidence for molecular peer-pressure: two sodium channel (NaCh) isoforms with different demeanors located in the axon initial segment (AIS), one of which is a bit cautious and the other is more impetuous. Neurons are continuously barraged by synaptic input that 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, 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 NaChs[2] and initiates action potentials[3]. Once initiated, spikes propagate in two directions: forward down the axon to cause neurotransmitter release by depolarizing presynaptic terminals[4] and backwards through the soma and then on to the dendrites. Although the forward-propagating action potential transmits information to downstream postsynaptic neurons, the backpropagating action potential enables forms of synaptic plasticity[5,6]. The unique characteristics of the AIS that allow it to both initiate spikes with relative ease and then guarantee subsequent backpropagation have remained elusive.

Here, Hu *et al.*[1] show, using quantitative immunostaining, electrophysiology (including the method of axonal bleb recording developed by one of the authors, Y. Shu) and computer modeling, that two NaCh subtypes, the high-threshold $Na_v1.2$ and the low-threshold $Na_v1.6$, are asymmetrically distributed in the AIS, precisely localizing these NaChs in the complex topography of the neuron. $Na_v1.2$ is found mainly in the 25 µm of the AIS that is 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 depolarization[7]. 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 that explains many of the unique properties of the AIS, including the faithful generation of backpropagating action potentials (**Fig. 1**).



Kim Caesar

Figure 1 A new blueprint for action potential initiation in the AIS. Excitatory neurotransmission onto the dendrites of a layer V pyramidal cell (green oval) causes depolarization of the postsynaptic V_m. This local depolarization moves electrotonically toward the soma (green arrow), 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 AIS (inset). Once incoming depolarization (green arrow) reaches the AIS (1), it will first enter an area rich in $Na_V1.2$ (blue). These channels are the 'cautious' high-threshold subtype, so depolarization will pass through without rapidly activating the $Na_V1.2$ channels (green arrow, masked by blue). When the wave of depolarization reaches the trigger-happy low-threshold $Na_V1.6$ 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 $Na_V1.6$ channels, causing a chain reaction of NaCh opening and initiating a forward-propagating action potential (AP, 3). Because $Na_V1.2$ channels were bypassed by the initial synaptic depolarization, they are available for activation, rather than being in an inactivated state. When $Na_V1.6$ channels open, they drive $Na_V1.2$ channel activation (4), inducing a secondary wave of inward sodium currents and initiating a backpropagating action potential (5). Because $Na_V1.6$ channels will be in their inactive state, $Na_V1.2$ channel opening will not induce a secondary forward-propagating action potential.

In this new model, action potentials are detonated by $Na_V1.6$ channels because of their low threshold for activation and high channel density[8]. $Na_V1.6$ channels sit in the perfect location to allow their easy initiation of action potentials: 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 $Na_V1.6$

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figures that 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 backpropagating action potential. 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 that are ready and waiting to initiate a backpropagating action potential. Furthermore, because $Na_V1.6$ channels in the distal AIS have entered an inactive state by the time $Na_V1.2$ channels open, a second forward-propagating action potential is prevented. Although elements of this scheme are not perfectly clear, this mechanism of spike initiation followed by faithful generation of a backpropagating action potential is both alluring and exciting.

The initiation of action potentials in the AIS is not a new concept. In fact the mechanism proposed by Hu *et al.*[1] draws on years of work from groups dedicated to understanding the specific mechanism of spike generation. It was initially reported over 50 years ago that the action potential appears first in the AIS of motoneurons and is followed by a backpropagating somatodendritic action potential[3]. As electrophysiological and imaging techniques advanced, so did our understanding of spike initiation. Pioneering studies[9] used simultaneous recording from the soma and AIS of subicular neurons to demonstrate that V_m rises more rapidly in the AIS during an action potential, which occurs presumably as a result of the $Na_V1.6$ localization found by Hu *et al.*[1]. In the soma, a previous study[9] showed that the onset of a spike occurs more slowly initially, resulting from what we now think is $Na_V1.6$-mediated depolarization in the distal AIS, and is then followed by a rapid increase in V_m which now appears to be driven by $Na_V1.2$ activation in the proximal AIS. This study[9] also showed that somatic action potential threshold is established by sodium channels ≈50 μm from the soma, where Hu *et al.*[1] have localized $Na_V1.6$. Although the authors of that study did not know the identity of the NaChs subtypes driving action potentials, they proposed the idea of a 'heminode' beyond the AIS where action potentials originate, an idea that is conceptually validated by Hu *et al.*'s[1] finding of a high concentration of $Na_V1.6$ channels in the distal AIS.

Recently, a study[10] unraveled the long–standing mystery of why previous recordings haven't revealed a higher density of NaChs in the AIS than elsewhere in the neuron if AIS NaCh density explains spike initiation. Answering this question required a literal deconstruction of the AIS, a structure that is notorious for its dense cytoskeleton[11]. The AIS is rich in the adaptor protein ankyrin G, which helps cluster both NaChs[12] and potassium channels[13]. It was demonstrated[10] that disruption of the actin cytoskeleton, and presumably its ability to stabilize ankyrin G, caused a threefold increase in the sodium current that could be recorded in the AIS of layer V pyramidal neurons[10]. This suggests that the AIS is indeed highly enriched in NaChs, but rigid cytoskeletal scaffolding somehow prevents ideal attachment of a patch pipette. 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 *et al.*[1] also address a recent controversy in the spike generation field: the possibility that NaCh activation is a cooperative process[14,15]. When action potentials 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.*[1] 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 were cooperative, one would expect that removing a subset of NaChs from the active pool of channels with tetrodotoxin would alter channel activation, whereas 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 action potentials and supports the idea that the rapid onset of action potentials in the soma results from recording distally from the site of action potential initiation.

Is there a new integrated view of spike initiation in pyramidal neurons? Hu *et al.*[1] combined their electrophysiological and immunohistochemical findings with elegant modeling experiments to confirm the roles of $Na_V1.6$ and $Na_V1.2$. By altering the relative amounts of $Na_V1.2$ and $Na_V1.6$ in their model, they found that the forward-propagating action potential threshold is almost completely dependent on the impulsive $Na_V1.6$, whereas the threshold for generating a backpropagating somatodendritic action potential is controlled by the hesitant $Na_V1.2$. It will be exciting to see which other unique biophysical parameters of $Na_V1.2$ and $Na_V1.6$ are relevant to additional aspects of spike generation and neuronal excitability. Will the faster recovery from inactivation seen in $Na_V1.2$ mean that they are more responsible for action potential generation during high-frequency firing? Or will the ability of $Na_V1.6$ to maintain high current amplitude during repeated activation put it in the driver's seat during high-frequency spiking[7]? Will the differential effects of drugs modulating NaCh properties (that is, phenytoin, carbamazepine, lamotrigine, etc.) be better understood now that we know more about the specific ion channels mediating action potential generation? With this detailed picture of the spike-generation machinery, we are much better equipped to answer these and other pressing questions.

Finally, our understanding of spike generation has truly paralleled our technical advances in electrophysiological and imaging techniques. From early intracellular recordings from motoneurons[3] to our ability to make simultaneous patch clamp recordings from a single neuron at multiple locations to *in vivo* recording of action potential threshold, our knowledge of spike initiation continues to grow. Now techniques such as voltage and sodium imaging and bleb recording are rapidly advancing our ability to characterize excitability in specific neuronal substructures. The most intriguing question that Hu *et al.*[1] leave unanswered is how is the 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 that have been so elegantly studied in the synapse now must be understood in the AIS.

1. Hu, W. *et al. Nat. Neurosci.* **12**, 996–1002 (2009).
2. Catterall, W.A. *J. Neurosci.* **1**, 777–783 (1981).
3. Coombs, J.S., Curtis, D.R. & Eccles, J.C. *J. Physiol. (Lond.)* **139**, 232–249 (1957).
4. Katz, B. *The Release of Neural Transmitter Substances* (Liverpool University Press, Liverpool, UK, 1969).
5. Markram, H., Lubke, J., Frotscher, M. & Sakmann, B. *Science* **275**, 213–215 (1997).
6. Magee, J.C. & Johnston, D. *Science* **275**, 209–213 (1997).
7. Rush, A.M., Dib-Hajj, S.D. & Waxman, S.G. *J. Physiol. (Lond.)* **564**, 803–815 (2005).
8. Kole, M.H. & Stuart, G.J. *Nat. Neurosci.* **11**, 1253–1255 (2008).
9. Colbert, C.M. & Johnston, D. A. *J. Neurosci.* **16**, 6676–6686 (1996).
10. Kole, M.H. *et al. Nat. Neurosci.* **11**, 178–186 (2008).
11. Song, A.H. *et al. Cell* **136**, 1148–1160 (2009).
12. Zhou, D. *et al. J. Cell Biol.* **143**, 1295–1304 (1998).
13. Pan, Z. *et al. J. Neurosci.* **26**, 2599–2613 (2006).
14. Naundorf, B., Wolf, F. & Volgushev, M. *Nature* **440**, 1060–1063 (2006).
15. McCormick, D.A., Shu, Y. & Yu, Y. *Nature* **445**, E1–E2 (2007).