activation[4,5]. Considering the importance of A_1R in regulating diverse physiological processes[9], it will be interesting to determine whether any of the non–pain-related effects of acupuncture are a result of A_1R activation.

Painful stimuli activate several brain regions, including the anterior cingulate cortex (ACC), with activation being proportional to the intensity and subjective perception of pain[10]. As acupuncture inhibited pain in the mouse behavioral models, this raised the question of whether pain-evoked activation in ACC might also be reduced. Indeed, both acupuncture and injection of CCPA into the mouse Zusanli point reduced pain-evoked activation in ACC. These inhibitory effects were also A_1R dependent. Notably, the A_1R agonist caused a rapid inhibition in the ACC, whereas acupuncture caused a more slowly developing inhibition. This latter finding is reminiscent of observations in humans, where acupuncture-induced pain relief is gradual, building up over time[1]. As speculated by Goldman *et al.*[2], the beneficial effects of acupuncture could reflect a slow buildup of extracellular adenosine.

There are two main ways that adenosine can reach the extracellular space[4,5]. Adenosine can be extruded from cells through nucleoside transporters or adenosine can be generated by ectonucleotidases, a class of enzymes that hydrolyze extracellular nucleotides to adenosine (**Fig. 1a**)[11]. Goldman *et al.*[2] found that ATP, ADP, AMP and adenosine were released following acupuncture (instead of just adenosine), suggesting an ectonucleotidase-dependent origin for adenosine. Indeed, the authors' experiments suggested that prostatic acid phosphatase, a recently identified ectonucleotidase[12], might hydrolyze AMP to adenosine in muscle near the Zusanli point. Ectonucleotidases inhibit nociception by generating adenosine and activating A_1R[12,13]. It will therefore be interesting to determine whether deletion or pharmacological inhibition of these enzymes blocks the pain-relieving effects of acupuncture.

Adenosine receptor signaling is ultimately terminated through a number of mechanisms. The ligand adenosine can be metabolized to inosine by adenosine deaminase or it can be transported into cells (**Fig. 1a**), lowering its extracellular activity. There are drugs that inhibit these processes, including deoxycoformycin (Pentostatin), an FDA-approved adenosine deaminase inhibitor. Because inhibition of adenosine metabolism generally prolongs the biological actions of adenosine[4,5], Goldman *et al.*[2] hypothesized that inhibition of adenosine deaminase might prolong the beneficial effects of acupuncture. Indeed, when deoxycoformycin injections were combined with acupuncture, the anti-nociceptive effects of acupuncture were extended by 2.0–2.5 h (**Fig. 1b**). This result provides additional validation that acupuncture acts via adenosine receptors. In addition, it suggests a pharmacological approach for enhancing the duration of acupuncture pain relief. Acupuncture, because of its short duration of action, is clinically most useful for the short-term management of pain[14]. By combining acupuncture with an FDA-approved drug such as Pentostatin, it might be possible to extend the utility of acupuncture to chronic pain conditions.

Lastly, in light of these new results, pain patients may want to lay off the coffee, tea or any other consumable with high levels of caffeine or theophylline. These stimulants block A_1R and block the anti-nociceptive effects of electroacupuncture in rats[15] (**Fig. 1b**). A typical cup of brewed coffee has ~100 mg caffeine, providing a dose of ~1.2 mg of caffeine per kg of body weight to the average person (weighing ~81 kg or 180 lbs). This dose is markedly similar to the 1.6 mg per kg dose used in a previous study in rats[15], so a cup of coffee may well reduce the pain-relieving effects of acupuncture or A_1R activity in general.

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The Na^+ channel conundrum: axon structure versus function

Daniel Johnston

High Na^+ channel density is thought to underlie the axon initial segment's (AIS's) low action potential initiation threshold, but a new study estimates functional Na^+ channel density in the AIS is only threefold higher than in the soma.

The questions of where is the action potential initiated in a neuron and why is it initiated there would seem to have been answered more than 50 years ago beginning with classic work on spinal motoneurons[1] and by subsequent theoretical and experimental work[2–4] (see also ref. 5). Ask this question to most any neuroscientist and they would almost certainly answer that the action potential is initiated in the axon hillock or initial segment because the threshold is lower there as a result of a higher density of sodium channels. This notion for action potential initiation is well entrenched in the psyche of most neuroscientists, but is it true? As surprising as it may seem, one of the more fundamental questions in neuroscience remains as controversial as ever. A new study by Fleidervish *et al.*[6] adds an important set of findings to this controversial issue and concludes that, although the action potential does appear to be initiated in the AIS of cortical pyramidal neurons, it is not because of a high density of sodium channels.

This dilemma stems in part from the distinction between structure and function. The structural evidence for a high density of sodium channels in the AIS is both abundant and consistent. Much of this evidence relies

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on immunohistochemistry (IHC), in which antibodies for sodium channels are attached to either a fluorophore or a gold particle for detection with a fluorescence or electron microscope. IHC is a powerful technique, but it must distinguish among the different types of sodium channels, such as $Na_v1.1$, 1.2 and 1.6, and between functional channels expressed on the surface and nonfunctional channels synthesized, packaged and transported inside the neuron. A recent study[7] used an impressive new method that combines freeze fracture, an improved fixation technique and immunogold electron microscopy to localize the different types of sodium channels on the surface of the membrane. The study argued that this method can account for every single sodium channel and concluded, again, that there is a high density of sodium channels in the AIS. Case closed?

Well, not quite. The functionalists or physiologists (full disclosure, I'm a physiologist) have a somewhat different view. Here the evidence is also consistent, although not quite so abundant or without exception[8]. Several studies using dual recordings of the axon and soma along with local application of the sodium channel blocker tetrodotoxin have confirmed the findings of the classic studies[1] that the threshold for action potential initiation is lower in the axon and that, under most conditions, the action potential initiates there. The studies that have diverged the most from the structural evidence, however, are those in which cell-attached or excised patch-clamp techniques have been used to record the number of active or functional sodium channels along the axon[8,9]. Quite surprisingly, these studies have not found the high density of sodium channels in the axon that has been suggested by both structural and theoretical evidence (estimated to be in the range of 30–1,000-fold higher in the axon than in the soma), and, in fact, the functional density was not much different in the axon from that in the soma. Some criticism has been raised against these patch-clamp experiments, however, because of the unique cytoskeleton of the AIS[8].

Fleidervish *et al.*[6] used a different approach for measuring functional sodium channels. They injected cortical pyramidal neurons with a dye that fluoresces when it binds to sodium ions and then used a high-speed camera to record changes in fluorescence during single and trains of action potentials. The method is similar to widely used techniques for calcium imaging, but sodium ions and the dyes that bind them are quite different from calcium ions and their respective fluorescent indicators. The most important difference is that, although calcium ions are highly buffered inside the cell, sodium ions are not; after entering through sodium channels, sodium ions are free to diffuse throughout the cell. The fluorescent indicator for sodium (sodium-binding benzofuran isophthalate) is thus able to detect all of the entering sodium ions and measure the resulting concentration changes in different compartments of the neuron.

These results led to several conclusions. First, there is a large rapid rise in sodium with a single action potential approximately 10–30 μm from the soma. This would be in the area of the AIS and consistent with the previous idea that the action potential initiates at this location. Second, the decay of the sodium concentration is rapid and unaffected by sodium pumps, even following trains of action potentials. This result is a bit surprising in that it suggests that the sodium concentration gradient between the inside and outside of the axon is maintained, at least in the short term, by energy-efficient diffusion rather than by energy-consuming active transport. Third, and what is likely to draw the most discussion and criticism, after accounting for the sodium ions entering the axon through open sodium channels, Fleidervish *et al.*[6] concluded that the density of functional sodium channels in the AIS is no more than threefold higher than in the soma, and not the 30–50-fold increase suggested by structural studies. In a few fortuitous and impressive experiments, they were even able to measure changes in sodium ion concentration at the first node of Ranvier, about 100 μm from the soma, and concluded that the sodium channel density is about the same in the node as in the AIS.

If the sodium channel density is similar in the AIS as in the soma, why is the threshold for action potential initiation lower in the axon? Fleidervish *et al.*[6] suggest that one contributing factor may be the type, or gating mode, of sodium channels present in the axon. They found a substantial increase in sodium ion concentration in the axon, but not in the soma, during subthreshold depolarizations. Some types of sodium channels have a persistent, or noninactivating, gating mode below the threshold for fast action potentials[10]. The results of Fleidervish *et al.*[6] would suggest that these channels are only present in the axon and that they may contribute to the lower threshold for the fast action potential by adding to the depolarization in the axon[11]. The authors also found, however, that even without this persistent sodium current in the axon, a lower threshold can be achieved with different voltage activation ranges for axonal sodium channels (see also refs. 12,13).

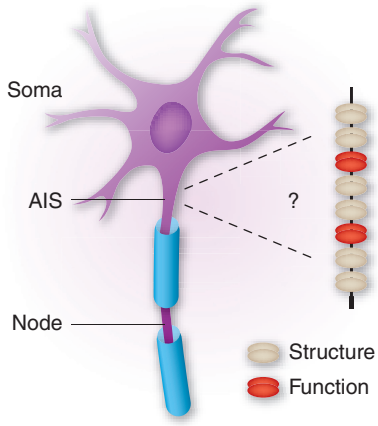

Figure 1 Schematic diagram for the dilemma between structural and functional evidence for the density of sodium channels (gray, red) in the AIS. The structural evidence suggests a high density of sodium channels in the AIS compared with the soma, whereas the data provided by Fleidervish *et al.*[6] suggest that the functional density is no more than threefold higher than in the soma. One resolution of these two types of studies may be that not all of the sodium channels in the AIS participate in action potential firing.

In summary, the study by Fleidervish *et al.*[6] certainly does not resolve the apparent paradox between the structural and functional studies for sodium channel densities in the axon of cortical pyramidal neurons (**Fig.1**). If anything, it adds to the mystery. It will be extremely interesting in future work to find out why there might be so many anatomically identifiable, but nonfunctional, sodium channels in the axon. Thankfully, there is still a role for both structuralists and functionalists in modern neuroscience.

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