

## SCIENTIFIC COMMENTARY

## A new HCN1 channelopathy: implications for epilepsy

**This scientific commentary refers to ‘Cation leak underlies neuronal excitability in an HCN1 developmental and epileptic encephalopathy’ by Bleakley *et al.* (doi: 10.1093/brain/awab145).**

Epilepsy is the most common neurological disorder in children, with a prevalence of approximately 0.5–1%.<sup>1</sup> Genetic variation leading to changes in voltage-gated ion channel expression and function is a common cause of neonatal and infantile epilepsy.<sup>2</sup> One particular channel that has been extensively linked to childhood epilepsies is the hyperpolarization-activated cyclic nucleotide gated (HCN) channel.<sup>1,3</sup> Four HCN channel subunits, HCN1–HCN4, have thus far been cloned.<sup>3</sup> Of these, HCN1 and HCN2 are highly expressed in the brain, where they assemble to form tetrameric channels (Fig. 1).

*De novo* mutations in the genes for HCN1 and HCN2 have been associated with developmental and epileptic encephalopathies (DEE) as well as with milder forms of epilepsy.<sup>1</sup> The M305L mutation, for example, which occurs in the fifth transmembrane segment of the HCN1 subunit (Fig. 1), has been identified in DEE patients and has been characterized in heterologous systems.<sup>4</sup> In this issue of *Brain*, Bleakley and co-workers<sup>5</sup> study the impact of this mutation on neuronal excitability by generating an HCN1 M305L knock-in mouse model, with some unusual and interesting results.

The patients identified thus far with the *de novo* HCN1 M305L mutation all carry the heterozygous variant, have frequent tonic-clonic seizures, and show severely abnormal development including visual deficits and bilateral hearing impairment. Their seizures are resistant to most drugs including phenobarbitone and can only be controlled by valproate.

Lamotrigine, a Na<sup>+</sup> channel inhibitor and potential HCN channel enhancer,<sup>6</sup> has been shown to increase seizure frequency.

Consistent with this phenotype, Bleakley and colleagues<sup>5</sup> showed that heterozygous HCN1 M305L knock-in mice displayed high amplitude interictal spikes in electrocorticography recordings, although spontaneous seizure activity was rarely observed. The mice were also hyperactive compared to wild-types, with learning impairments and increased anxiety. Moreover, lamotrigine increased interictal spike activity in heterozygous HCN1 M305L knock-in mice, similar to the phenomenon observed in patients. The mouse model thus recapitulates a large proportion of the patient phenotype.

Using their model, the authors then examined the effect of heterozygous expression of HCN1 M305L on neocortical and hippocampal CA1 pyramidal neurons. Larger amplitude HCN1 channel currents were seen in neurons from HCN1 M305L knock-in mice compared to those from controls, with more HCN1 channels open at rest. As HCN1 channels are cation channels that are permeable to Na<sup>+</sup> and K<sup>+</sup> ions,<sup>3</sup> this should result in greater influx of Na<sup>+</sup> at rest and thus a more depolarized resting membrane potential. And indeed, a more positive resting membrane potential was observed in HCN1 M305L knock-in neurons compared with wildtype cells, and consequently, a given depolarization had a greater propensity for generating action potentials.

However, wild-type HCN1 channels are also slow to activate and inactivate.<sup>6</sup> Hence, injection of subthreshold hyperpolarizing or depolarizing currents generates a so-called 'sag' (Fig. 1). Moreover, the membrane resistance is then reduced. Unusually, the HCN1 M305L knock-in resulted in HCN1 channels that activated much faster than wildtype HCN1 channels. Consequently, the sag was reduced, though the membrane resistance remained unchanged. A reduction in membrane resistance by HCN1 channels causes a decrease in

excitatory synaptic potential (EPSP) amplitude and integration (Fig. 1).<sup>7</sup> An interesting question is whether HCN1 M305L subunit knock-in affects EPSP integration per se. Would the depolarized resting membrane potential be sufficient to influence EPSP–spike coupling and increase neuronal action potential firing *in vivo*? Moreover, HCN1 channels are highly expressed in neuronal dendrites,<sup>8</sup> which receive the most synaptic inputs. The effects of the HCN1 M305L subunit knock-in on dendritic excitability, and whether this affects neuronal action potential firing would thus be worth exploring.

HCN1 M305L knock-in altered the expression of two sodium channel subunits, SCN2A and SCN8A. However, there may also have been changes in the expression of other genes, which might alter the effect of the HCN1 M305L knock-in on neuronal excitability under physiological conditions *in vivo*. Furthermore, HCN1 channels can also be present in synaptic terminals and axons, where they regulate synaptic release and action potential propagation, respectively.<sup>3</sup> It would thus be interesting to determine how the HCN1 M305L knock-in affects these processes and thereby neural network excitability.

Nonetheless, the generation of the HCN1 M305L knock-in mouse model is a first and important step in understanding how this mutation could lead to neural network hyperexcitability and epilepsy. This mouse model may be a useful tool in assessing novel treatments for HCN1 channelopathies that are associated with epilepsy. Since many patients with HCN1 channelopathies have a poor response to current drug treatments,<sup>1</sup> finding new treatments is imperative for improving the lives of these individuals.

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## Competing interests

The author reports no competing interests.

## Figure

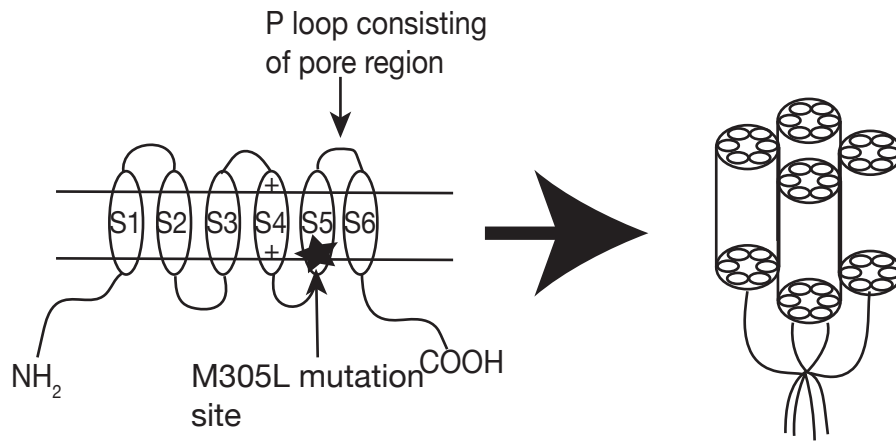
**Figure 1** (A) Schematic of an HCN1 channel subunit illustrating the position of the M305L mutation. Four HCN1 subunits come together to form an HCN1 channel as shown. (B) CA1 pyramidal neuron. Electrophysiological recordings can be obtained from the soma and dendrites (arrows). (C) Electrophysiological recording from the soma of a wildtype CA1 pyramidal neuron at the resting membrane potential ( $-71$  mV). Application of hyperpolarization and depolarization steps generates a sag due to activating and de-activating HCN channels. [D(i)] Electrophysiological recordings from cortical neuron dendrites of wild-type and HCN1 null mice at the resting membrane potential ( $-70$  mV). Loss of HCN1 channels (HCN1<sup>-/-</sup>) results in an increase in EPSP amplitude and wider EPSPs. [D(ii)] Multiple EPSPs summate to a greater extent in the absence of HCN1 channels than in wild-type (wt) neurons (adapted from Huang *et al.*<sup>7</sup>).

## References

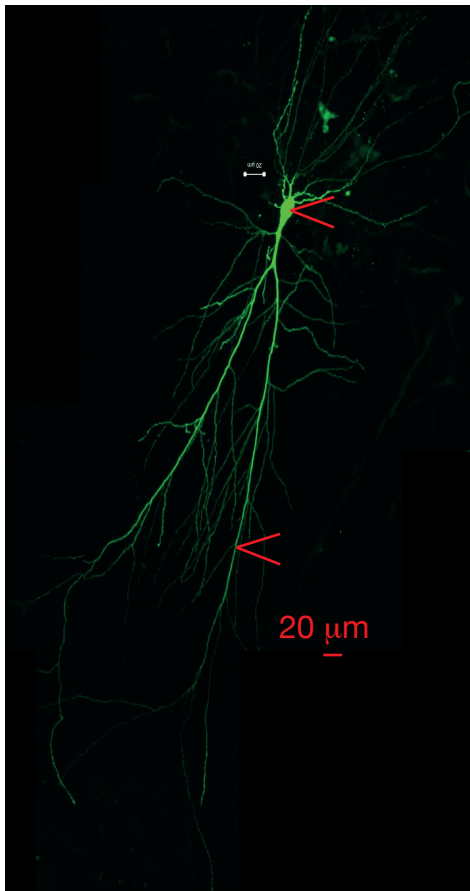
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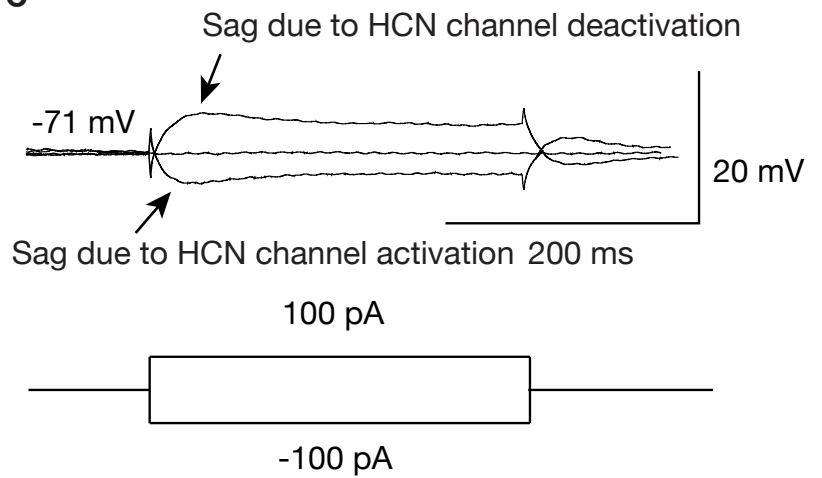
### A HCN1 channel Subunit



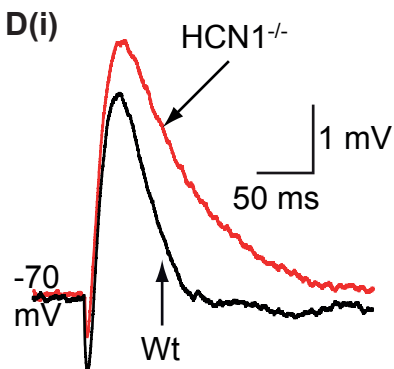
### B



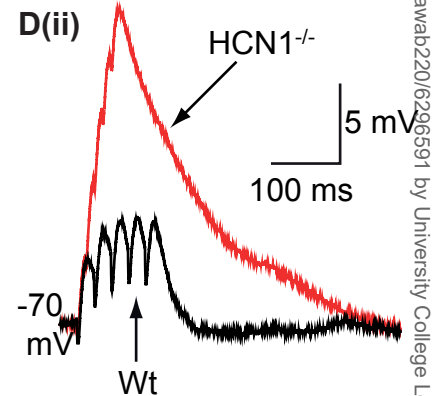
### C



### D(i)



### D(ii)



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