Electrophysiology of SCN8A Mutant Channels

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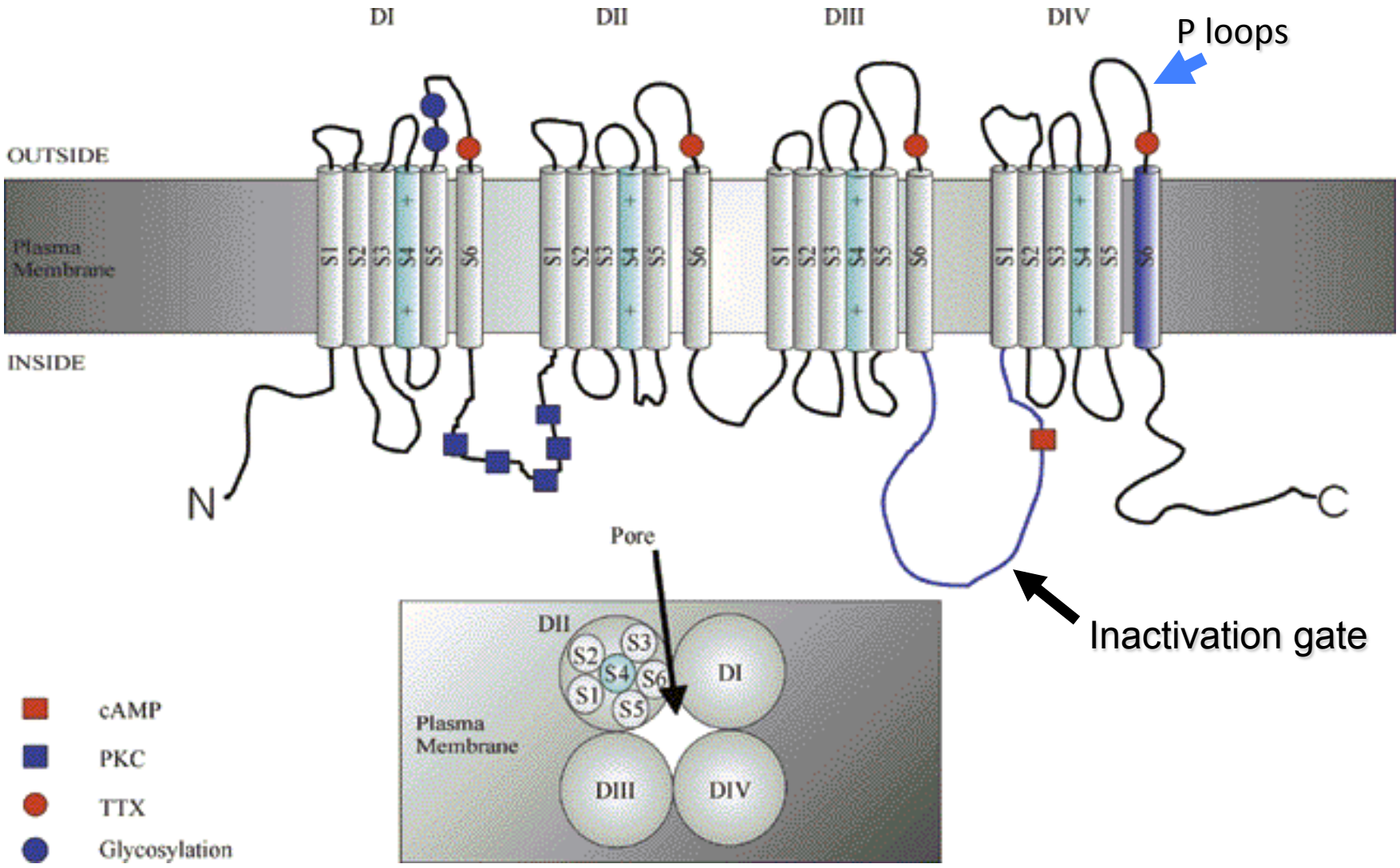
Outline

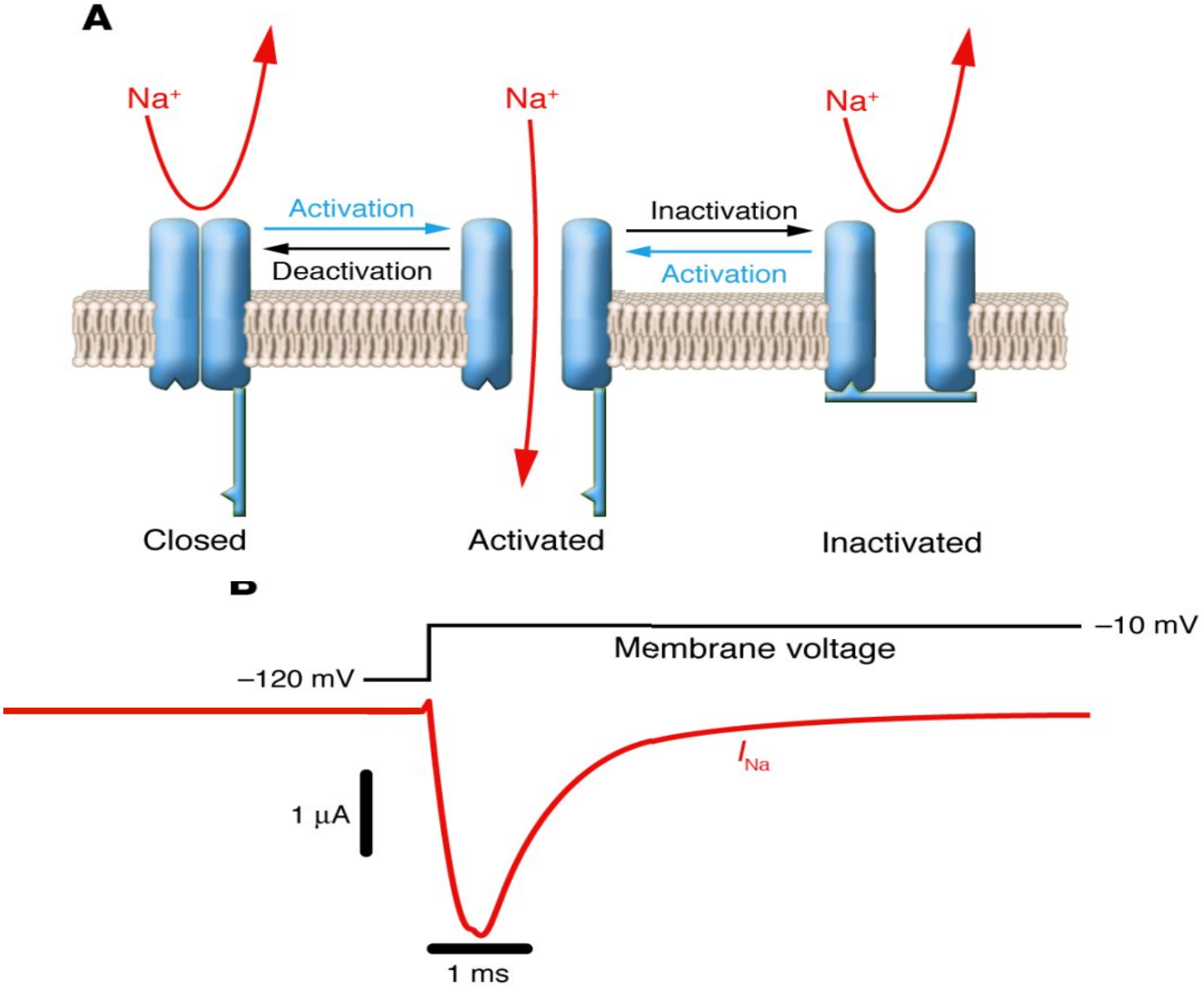
- Gain of Function Mutations
- Loss of Function Mutations
- Alterations in Nav1.6 in TLE
- Would knowledge of biophysical properties help with therapy?

Important structures of the Na channel



Adapted from Anger T., J Med Chem. 2001; 44:115-137

Normal Function of Sodium Channel

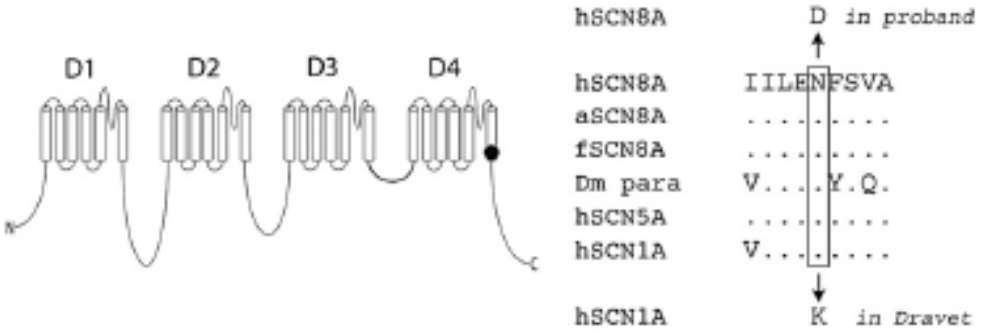


J. Clin. Invest. ***115****(8): 1990-1999 (2005)*

De Novo Pathogenic *SCN8A* Mutation Identified by Whole-Genome Sequencing of a Family Quartet Affected by Infantile Epileptic Encephalopathy and SUDEP

Krishna R. Veeramah,[1] Janelle E. O'Brien,[4] Miriam H. Meisler,[4] Xiaoyang Cheng,[5] Sulayman D. Dib-Hajj,[5] Stephen G. Waxman,[5] Dinesh Talwar,[6,7,9] Santhosh Girirajan,[10] Evan E. Eichler,[10] Linda L. Restifo,[2,7,8] Robert P. Erickson,[3,6] and Michael F. Hammer[1,*]

The American Journal of Human Genetics *90*, 502–510, March 9, 2012

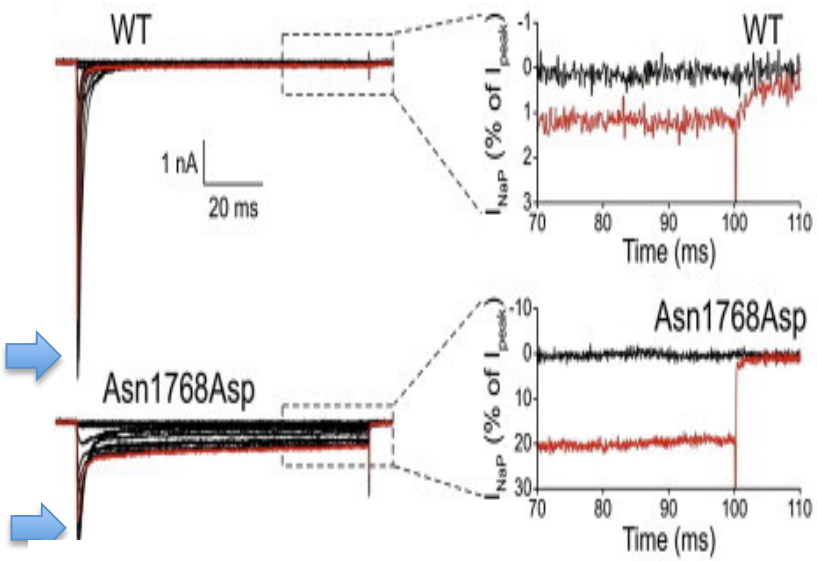


Asparagine to Aspartate; N1768D

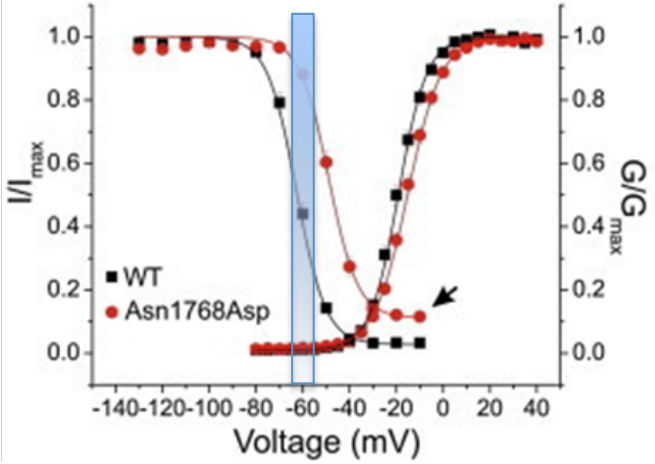
Gain of Function Mutation

Hyperexcitability due to impaired channel inactivation in EE *SCN8A* mutation p.ASN1768Asp.

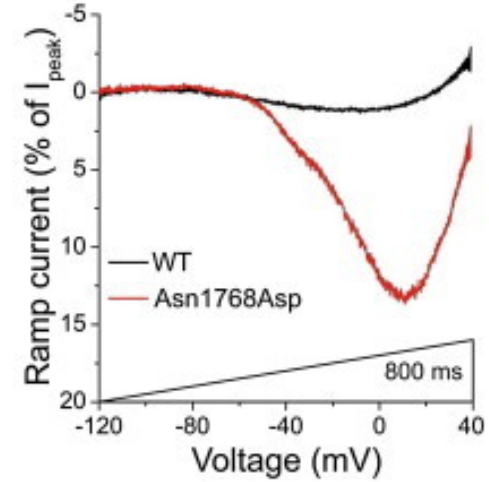
Persistent current activity



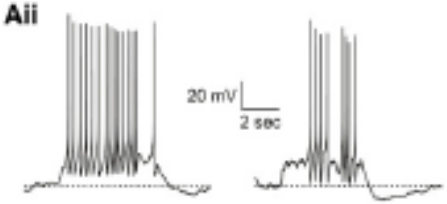
C Impaired Inactivation, but not **Activation**



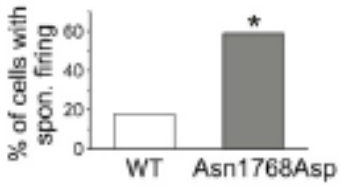
E Increase in non-inactivated channels

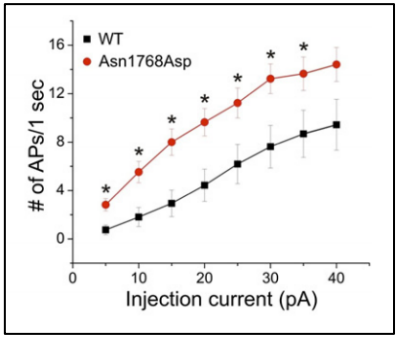


Aii



B

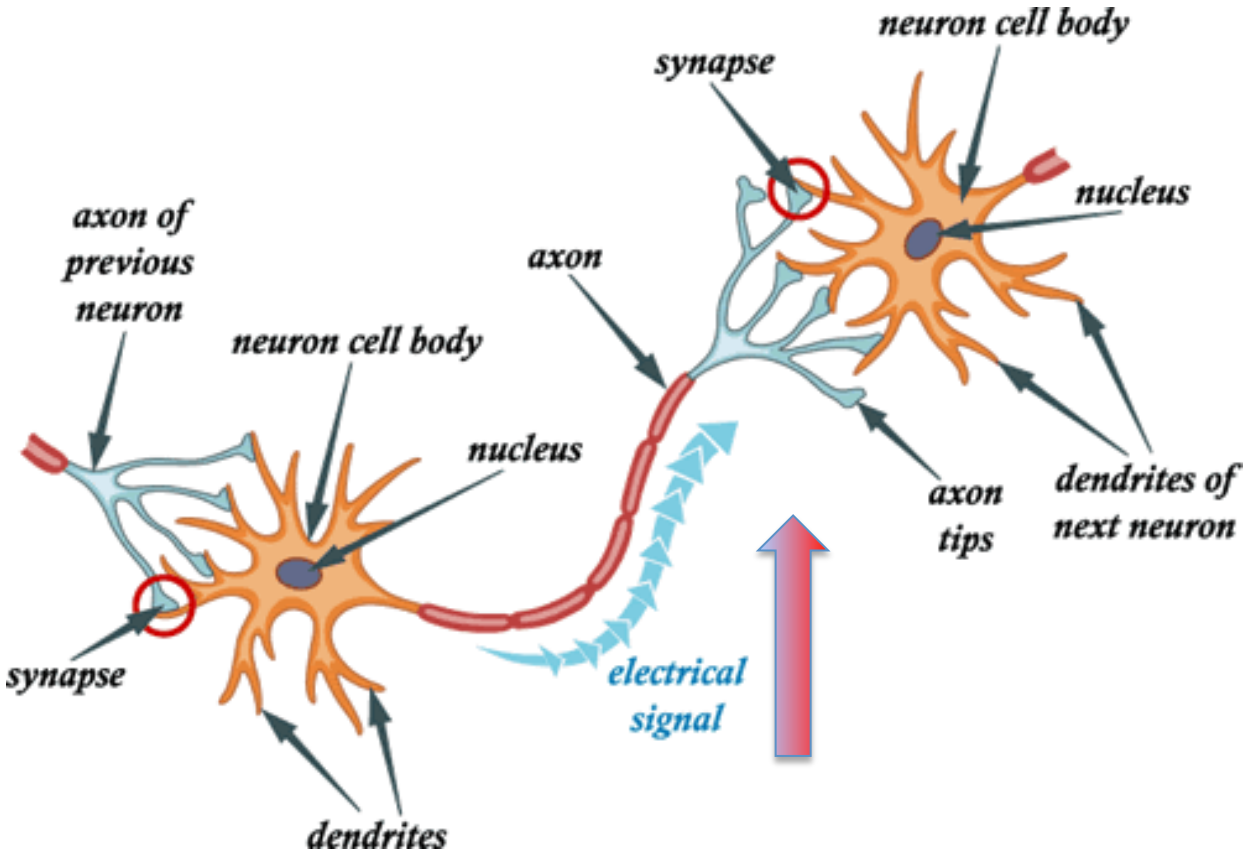




Increase in spontaneous firing.

Veeramah *et al*, AJHG 2012

Neuronal Networks Enhanced

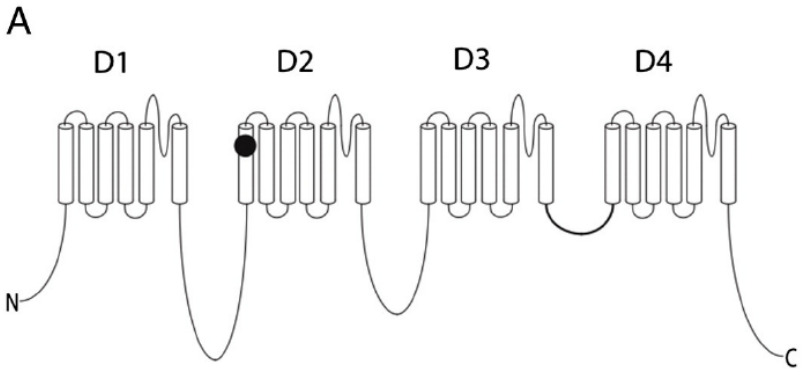


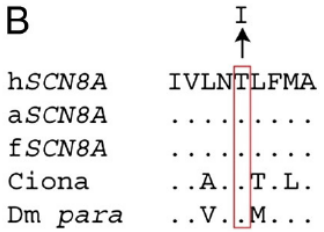
http://www.mysearch.org.uk

A novel de novo mutation of *SCN8A* ($Na_v1.6$) with enhanced channel activation in a child with epileptic encephalopathy

Mark Estacion [a,b], Janelle E. O'Brien [c], Allison Conravey [d], Michael F. Hammer [e], Stephen G. Waxman [a,b], Sulayman D. Dib-Hajj [a,b,*], Miriam H. Meisler [c,**]

Neurobiology of Disease 69 (2014) 117–123



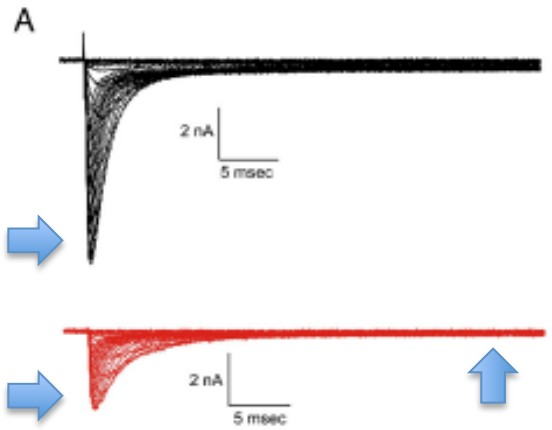


p.Thr767Ile
Threonine (polar) – Isoleucine (bulky hydrophobic).

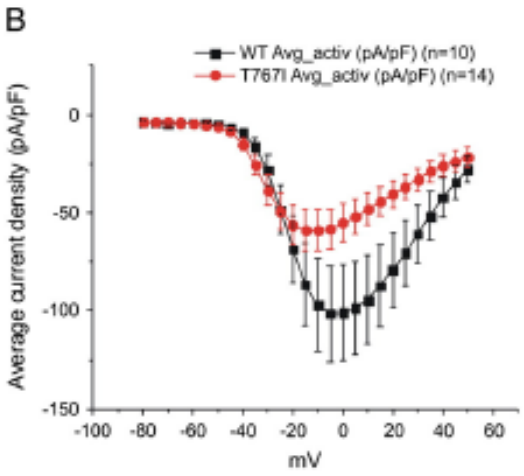
Proband exhibits profound developmental delay, intellectual disability and intractable epilepsy.

Gain of function mutation

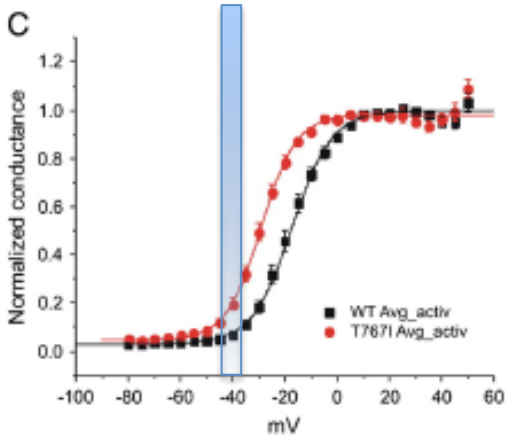
Current density is reduced by 2.5 fold



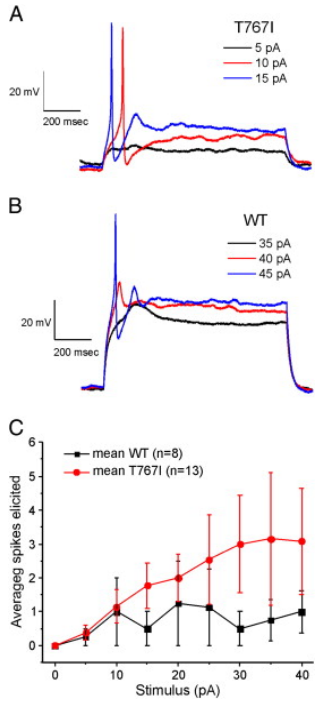
No persistent current



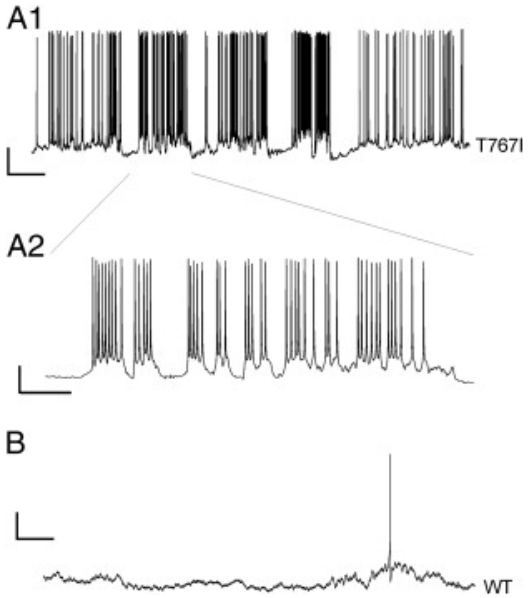
Hyperpolarized shift in activation



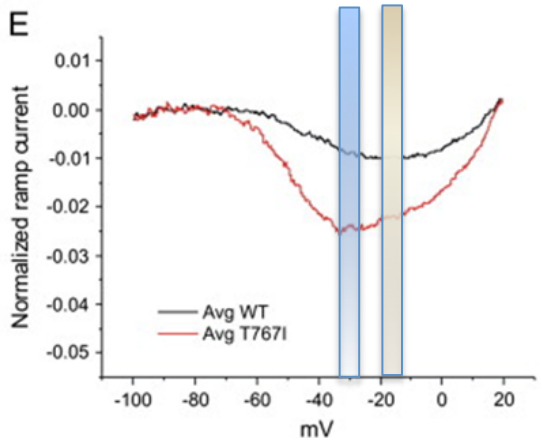
Reduced threshold



Increase in spon. activity



Ramp currents increased 3 fold



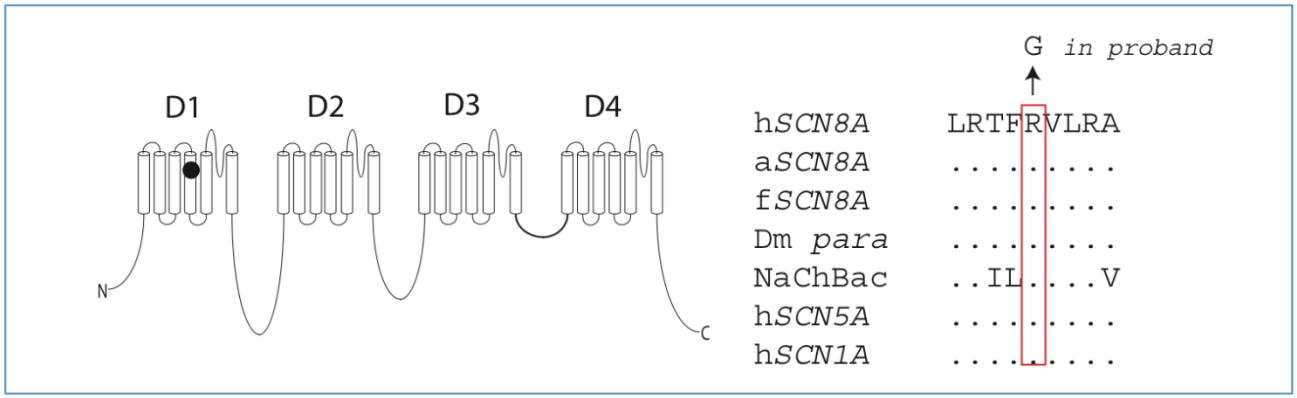
Neurobiology of Disease, Volume 69, 2014, 117 - 123

Characterization of a *de novo SCN8A* mutation in a patient with epileptic encephalopathy

Carolien G.F. de Kovel[a,*], Miriam H. Meisler[c,d], Eva H. Brilstra[a], Frederique M.C. van Berkestijn[b], Ruben van 't Slot[a], Stef van Lieshout[a], Isaac J. Nijman[a], Janelle E. O'Brien[c], Michael F. Hammer[g], Mark Estacion[e,f], Stephen G. Waxman[e,f], Sulayman D. Dib-Hajj[e,f], Bobby P.C. Koeleman[a]

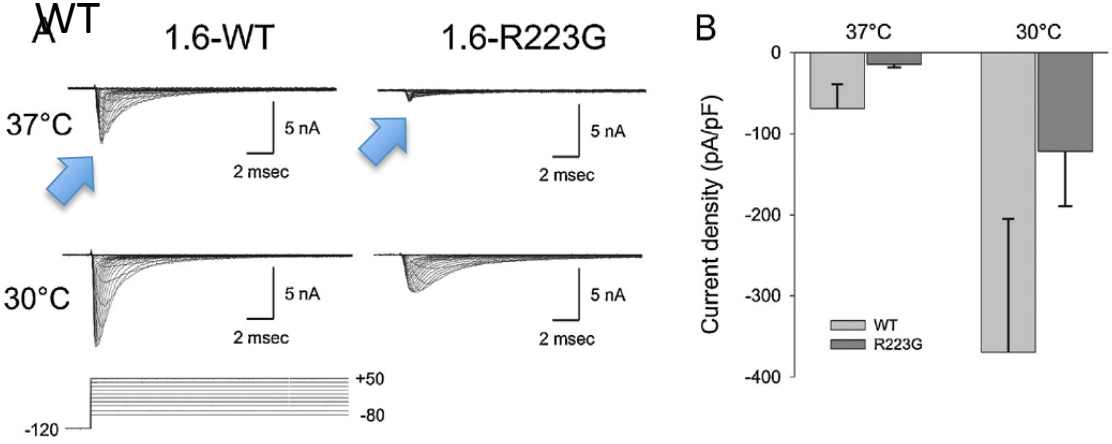
Epilepsy Research (2014) 108, 1511–1518

Arginine (R) – Glycine (G)

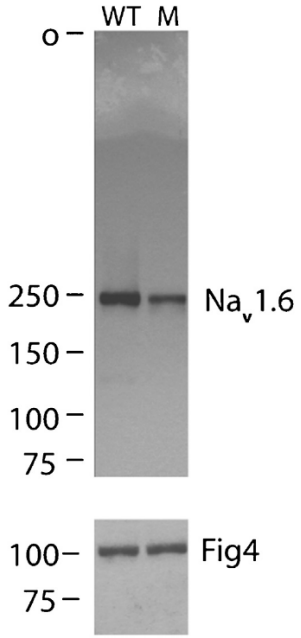


Loss of Function – clinical progression more severe – inhibitory neurons?

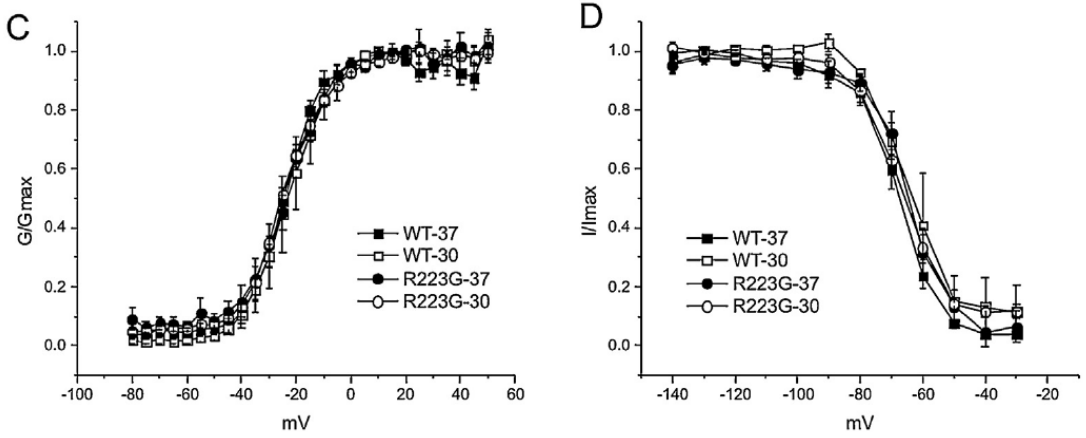
Reduced current density of mutant compared with WT



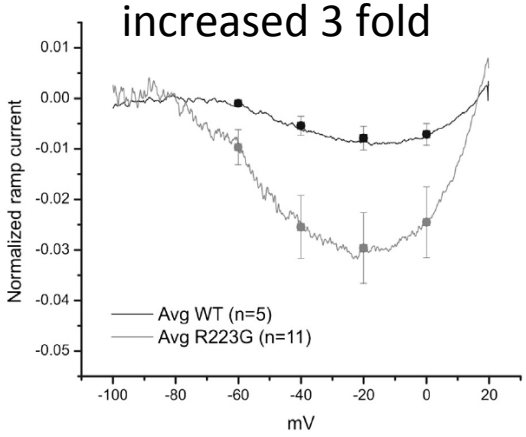
80% reduction in channel protein



Activation and Inactivation parameters are unaltered

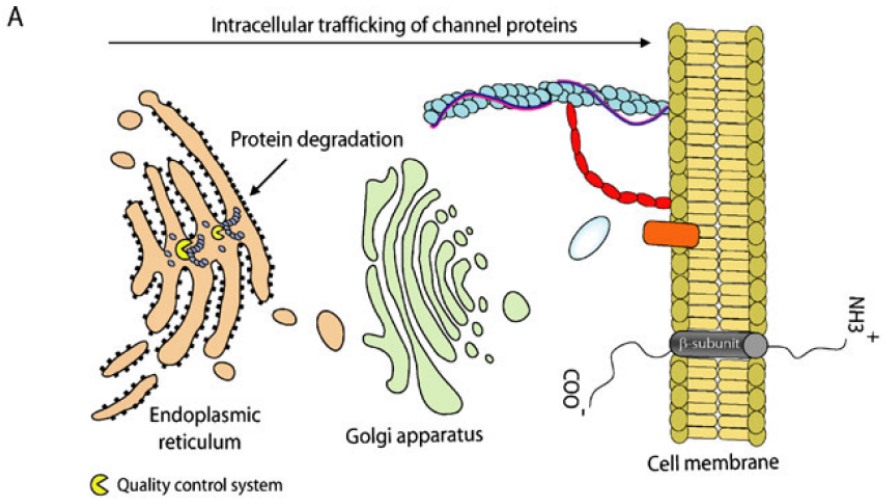


Ramp currents are increased 3 fold



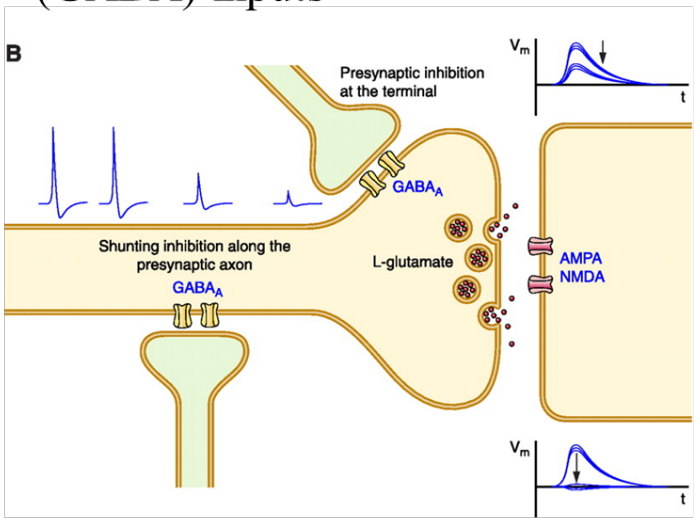
Reduction in activity of Inhibitory neurons as well as Excitatory neurons

Misfolded protein targeted for degradation



Pflugers Arch - Eur J Physiol (2010) 460:223–237

Inhibition of excitatory output by activation of pre-synaptic inhibitory (GABA) inputs



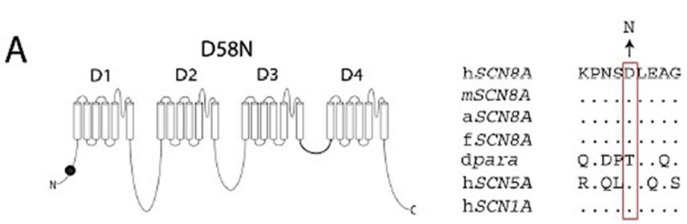
Pre - synaptic Post

Zeilhofer H U et al. Physiol Rev 2012;92:193-235

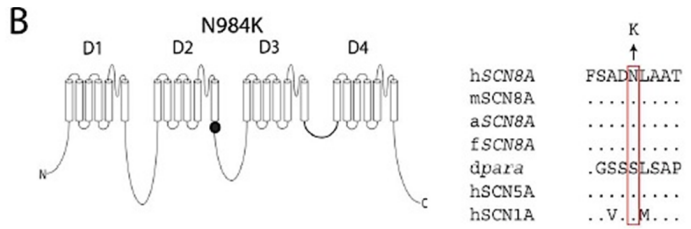
De novo gain-of-function and loss-of-function mutations of *SCN8A* in patients with intellectual disabilities and epilepsy

Maxime G Blanchard,[1] Marjolein H Willemsen,[2] Jaclyn B Walker,[3] Sulayman D Dib-Hajj,[4,5] Stephen G Waxman,[4,5] Marjolijn CJ Jongmans,[2] Tjitske Kleefstra,[2] Bart P van de Warrenburg,[6] Peter Praamstra,[6] Joost Nicolai,[7,8] Helger G Yntema,[2] René JM Bindels,[1] Miriam H Meisler,[3] Erik-Jan Kamsteeg[2]

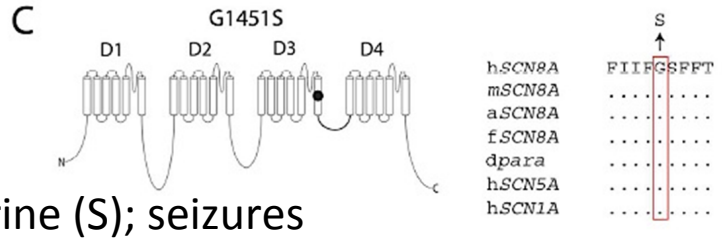
J Med Genet 2015;**52**:330–337.



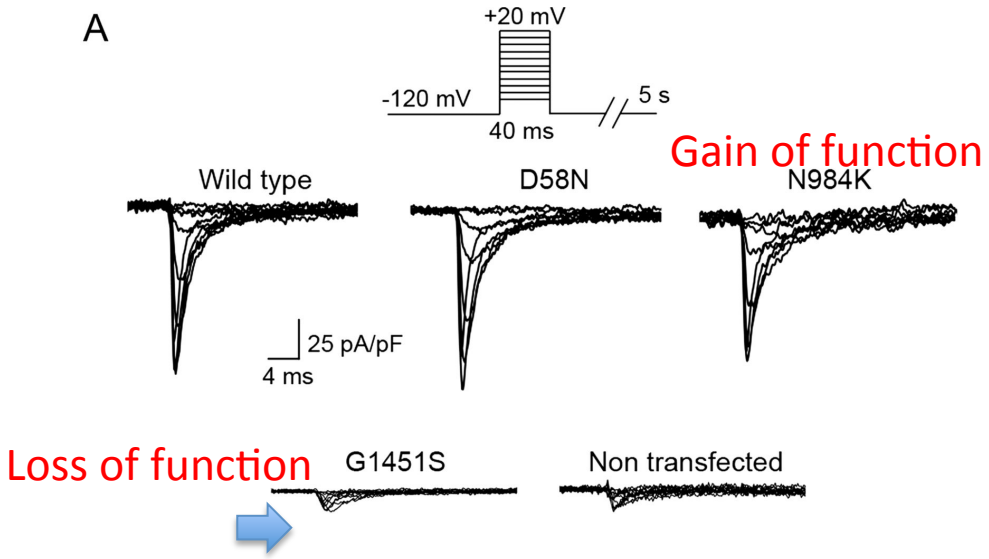
Aspartic acid (D) – Asparagine (N)
No seizures

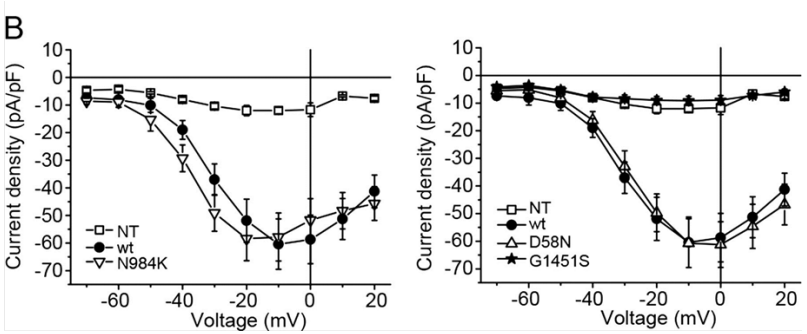


Asparagine (N) – Lysine (K)
Seizures

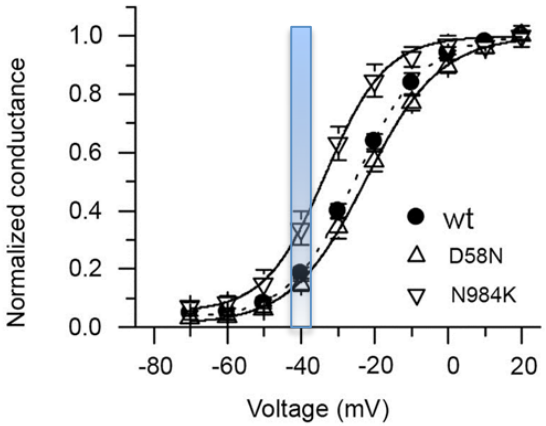


Glycine (G) – Serine (S); seizures

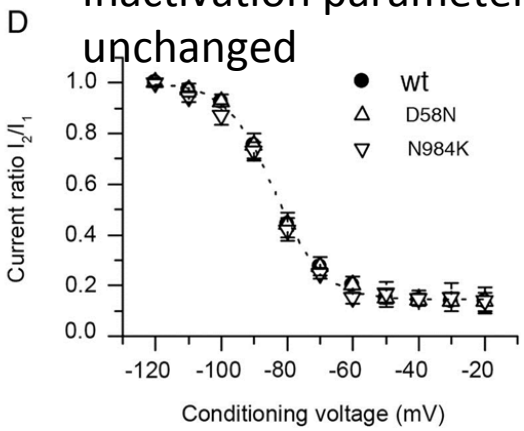




Activation parameters shifted in hyperpolarizing direction



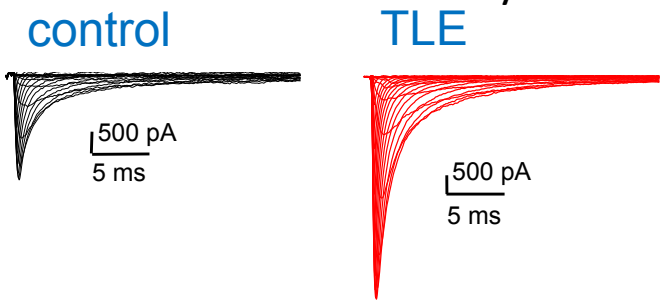
Inactivation parameters unchanged

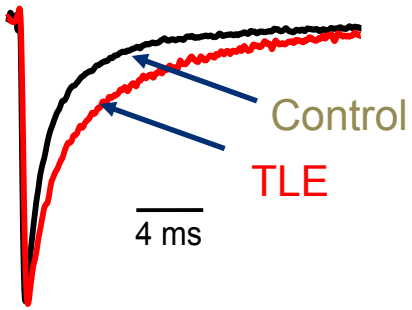


Maxime G Blanchard et al. J Med Genet 2015;52:330-337

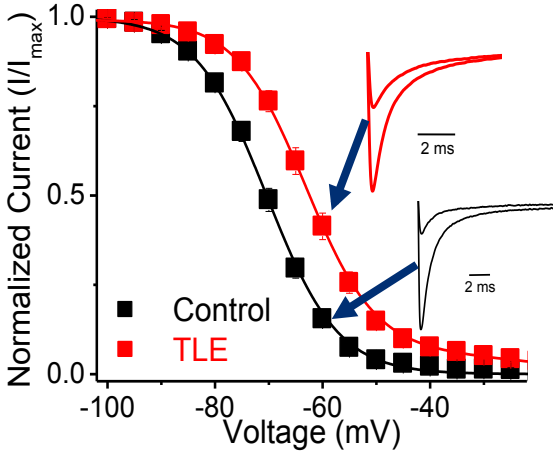
Nav1.6 in TLE

Increase in current density

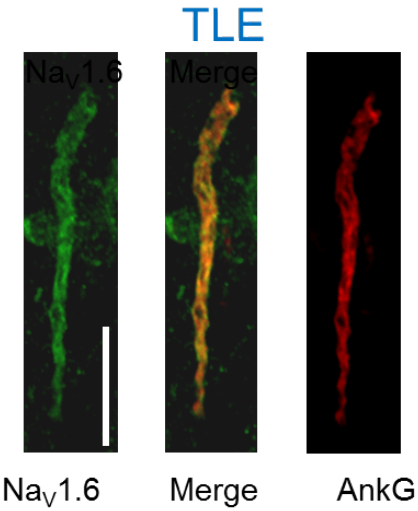


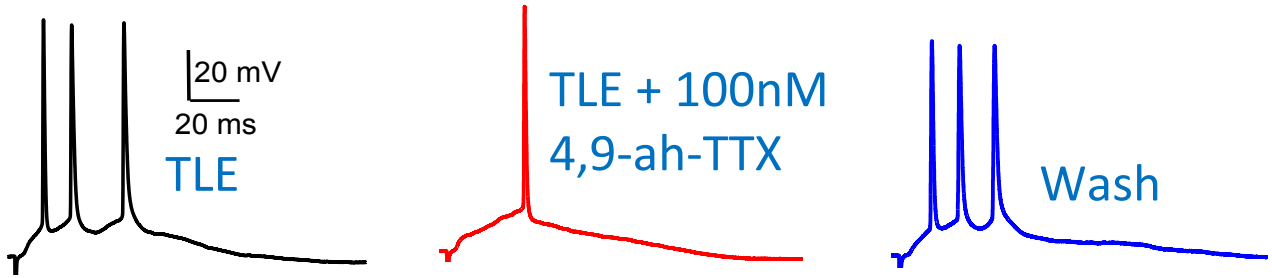


Delays in inactivation



Increased expression at AIS

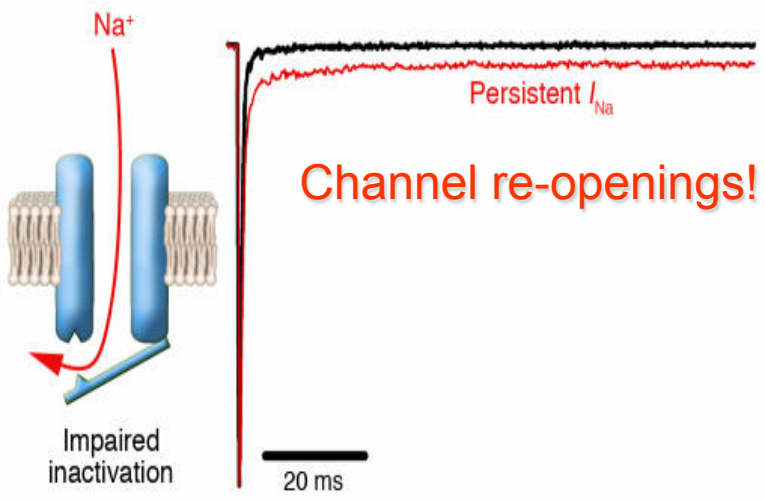




Block of 50% Nav1.6 reduces excitability

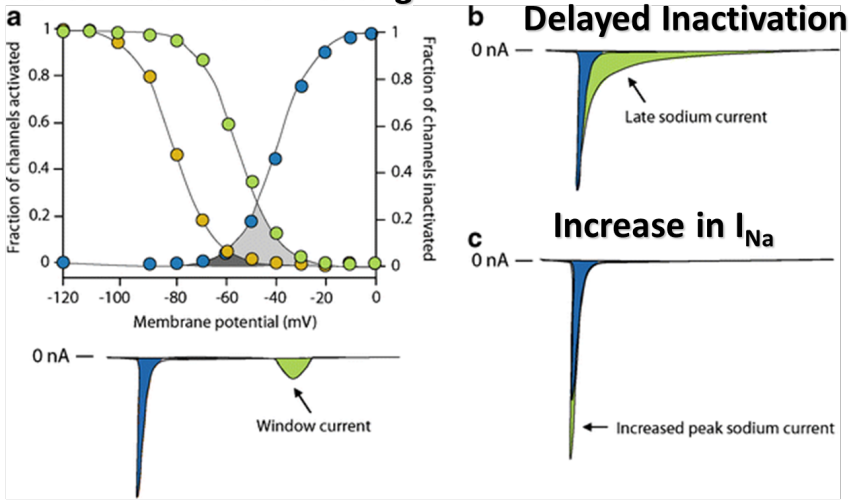
Hargus NJ, et al, J Neurophysiol. 2013; 110(5):1144-57.

Gain of Function

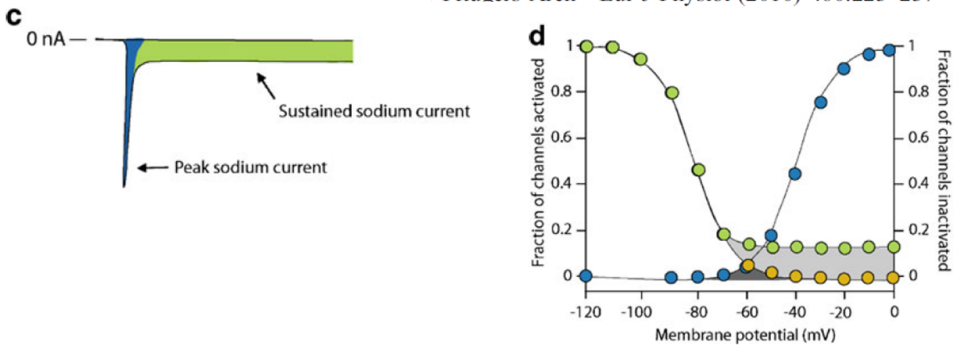


J Clin Invest. 2005 August 1; 115(8): 1990–1999

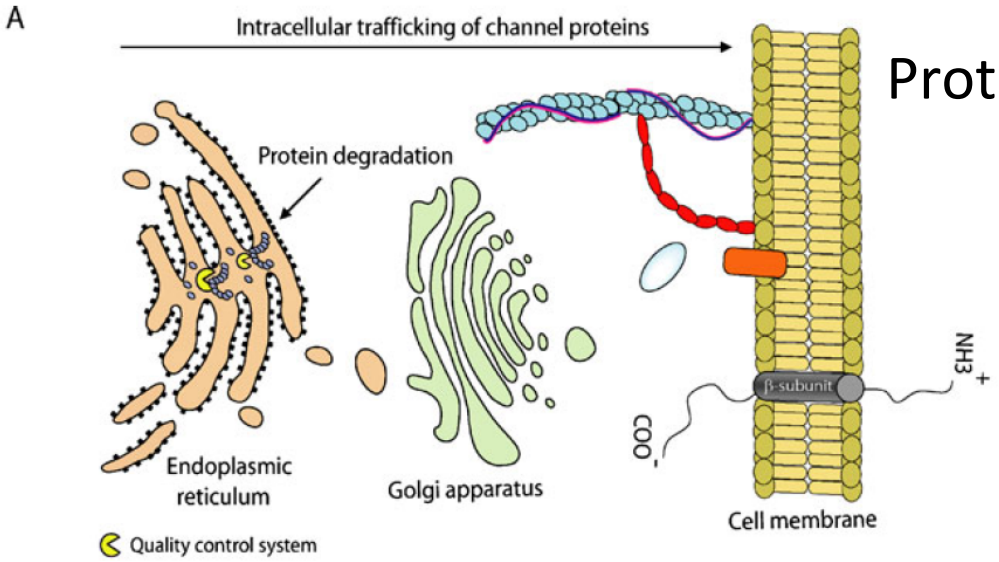
Altered Inactivation Gating



Pflugers Arch - Eur J Physiol (2010) 460:223–237

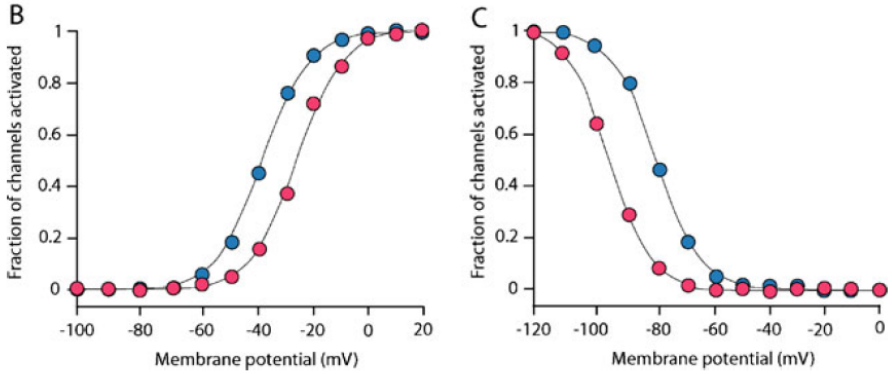


Loss of Function



Protein degradation – quality control

Delayed activation/ increased inactivation



Pflugers Arch - Eur J Physiol (2010) 460:223–237

Would knowledge of the biophysical properties help with treatment?

Practical Result: Avoidance of treatment with sodium channel blockers, which exacerbate symptoms in patients due to reduced expression of sodium channels