effectively degrading a well-confined window conductance into a TTX-sensitive ''Na+-leak''.
To assess whether minor membrane trauma could lead to Na+ (and hence Ca2+) loading of axons, we model partially left-shifted Nav operation in a free-running human node of Ranvier. Included are Kv and Nav conductances (linear and electrodiffusion driving forces, respectively) and a Na/K pump, with [ion]s (and associated ENa and EK) in realistic-sized intra- and extracellular compartments changing due to net ion fluxes. Left-shifting a fraction of the Navs immediately triggers a damped action potential burst then a voltage plateau dominated by window current. If resting conductances are large enough, pumping restores the system for several minutes, then more bursting starts and more ENa rundown occurs.

1613-Pos
Modulation of Nav 1.5 Variants by Src Tyrosine Kinase
Bagavananthem Andavan, **Gowri Shankar**, Rosa Lemmens-Gruber.
University of Vienna, Wien, Austria.
Cardiac sodium channel (Na_v 1.5) splice variant Q1077 deleted (hH1c1) and Q1077 present (hH1c3) mutants are present in 45% and 25 % of human population. In previous studies, Src tyrosine kinase (Fyn) showed opposite effects on cardiac and neuronal sodium channel inactivation. Half maximum inactivation of cardiac sodium channels was shifted to more positive potentials, whereas in neuronal channels it was shifted into the hyperpolarizing direction, despite having conserved Y1495, which is the site of phosphorylation in both channels. Activation was not affected.
In our study we found that Fynhas a different action on the cardiac sodium channel variants hH1c1 and hH1c3. Experiments were performed by means of the patch clamp technique in the whole cell mode. Fyn was transiently expressed with CD8 in stably expressed HEK293 cells embodying hH1c1 and hH1c3 clones. In hH1c1, Fyn shifted the activation (V_{mid} -51.6 $\pm$ 1.5 to -63.9 ± 1.0) and inactivation curves (V_{mid} -64.4 $\pm$ 0.7 to -72.5 ± 0.4) to more negative potentials, which could be reversed by the kinase inhibitor PP2 (activation: V_{mid} -63.9 ± 1.0 to -52.0 ± 1.9, and inactivation: V_{mid} -72.5 ± 0.4 to -63.7 ± 0.7). In contrast, in hH1c3 Fyn shifted both activation (V_{mid} -86.2 $\pm$ 2.3 to -65.8 ± 0.5) and inactivation (V_{mid} -85.1 ± 0.7 to -63.4 ± 0.3) curves to more positive potentials. PP2 reversed the shift of both, activation (V_{mid} -63.4 ± 0.3 to -88.2 ± 1.0)and inactivation (V_{mid} -63.4 ± 0.3 to -87.2 ± 1.7).
Above result proclaims that hH1c1 and hH1c3 encoding for Nav 1.5 are differently regulated by Fyn. These data will be pertinent in understanding the role of Q1077, which is present in the transport associated region that plays a pivotal role in regulating Fyn function.

1614-Pos
Calmodulin Regulation of the Neuronal Voltage-Dependent Sodium Channel
Michael D. Feldkamp, Liping Yu, Madeline A. Shea.
Univeristy of Iowa, Iowa City, IA, USA.
Calmodulin (CaM) is an essential eukaryotic calcium sensor comprised of two homologous domains (N, C). Ca^{2+} binding to CaM changes its conformation and determines how CaM recognizes and regulates target proteins such as the neuronal voltage-dependent sodium channel ($Na_v1.2$) which is essential for the generation and propagation of action potentials. $Na_v1.2$ is a multimer with one pore-forming α-subunit and one or more β-subunits. CaM binds to an IQ-motif (IQxxxBGxxxB, B=K,R) of $Na_v1.2$ that is near the C-terminus of the α-subunit. Prior thermodynamic studies showed that this IQ peptide ($Na_v1.2_{IQp}$, KRKQEEVSAIVIQRAYRRYLLKQKVKK) selectively lowers the Ca^{2+}-binding affinity sites in the C-domain of CaM, without affecting the N-domain (Theoharis et al, *Biochemistry* 2008). This selective decrease correlates with $Na_v1.2_{IQp}$ having a higher affinity for apo CaM than for calcium-saturated CaM. Structural studies of complexes of CaM bound to target peptides or proteins demonstrated that the 4-helix bundle of the CaM C-domain adopts an ''open'' conformation when Ca^{2+}-saturated. There is only one high-resolution structure (2IX7) of apo CaM bound to an IQ motif; it shows the C-domain having a ''semi-open'' conformation. To understand the Ca^{2+}-dependent conformational switching CaM when regulating $Na_v1.2$, we applied heteronuclear NMR methods. Amide exchange, hnNOE, and chemical shift perturbation experiments revealed residue-specific changes consistent with a ''semi-open'' conformation of the apo C-domain of CaM when bound to $Na_v1.2_{IQp}$. NMR experiments are complete and analysis is underway to determine the solution structure of the apo C-domain of CaM bound to $Nav1.2_{IQp}$. Understanding the interface between CaM and the IQ-motif of the channel will result in a more complete model of how CaM regulates $Na_v1.2$ function at low physiological [Ca^{2+}] in neuronal tissues. NIH GM57001

1615-Pos
Electrophysiological Characteristics of Neonatal Nav1.5 Expressed in a Highly Invasive Human Breast Cancer Cell Line: Sensitivity to pH and Divalent Cations
Mustafa B. Djamgoz, Rustem Onkal.
Imperial College London, London, United Kingdom.
Electrophysiological recordings from human carcinoma cell lines have shown consistently that strongly metastatic cells express functional voltage-gated sodium channels (VGSCs). The predominant VGSC in metastatic breast cancer, in vitro and in vivo, is the 'neonatal' splice form of Nav1.5. In this developmentally regulated D1:S3 splice variant of Nav1.5, there are 31 nucleotide differences between the 5'-exon ('neonatal') and the 3'-exon ('adult') forms, resulting in 7 amino acid differences in D1:S3-S3/S4 linker. In particular, a conserved negative aspartate residue in the 'adult' is replaced with a positive lysine. 'Neonatal' and 'adult' Nav1.5 α-subunit splice variants were stably transfected into EBNA-293 cells and their electrophysiological properties were investigated by whole-cell patch-clamp recording. Compared with the 'adult' isoform, the 'neonatal' channel exhibited (1) depolarized threshold of activation and voltage at which current peaked; (2) much slower kinetics of activation and inactivation; (3) ~50% greater transient charge (Na^+) influx; (4) a slower recovery from inactivation; and (5) larger persistent Na^+ currents. Mutating the lysine in the 'neonatal' channel back to aspartate resulted in the electrophysiological parameters studied reverting strongly back towards the 'adult', i.e. the lysine residue was primarily responsible for the electrophysiological differences. The charge difference between the two Nav1.5 isoforms was 'challenged' by H^+ and Cd^{2+}. The main differential effect occurred at pH 5.25-5.75 in which the activation parameters of 'neonatal' Nav1.5 were affected significantly less. The biophysical characteristics of 'neonatal' Nav1.5 observed could have significant developmental and pathophysiological consequences. In particular, the prolonged Na^+ influx can alter intracellular Ca^{2+} and/or pH homeostasis, at least in microdomains, and channel activation remains relatively efficient under extreme acidosis.

1616-Pos
Sodium Channel Variants Associated with Atrial Fibrillation Exhibit Abnormal Fast and Slow Inactivation
Dao W. Wang, Nila Gillani, Dan M. Roden, Dawood Darbar,
Alfred L. George.
Vanderbilt University Medical Center, Nashville, TN, USA.
Mutations and rare genetic variants in *SCN5A*, the gene encoding the cardiac voltage-gated sodium channel $Na_V1.5$, have been associated with inherited predisposition to ventricular arrhythmia. More recently, *SCN5A* variants have been identified in families segregating atrial fibrillation. We evaluated the biophysical properties of seven novel *SCN5A* variants associated with atrial fibrillation identified by our previous genetic study to elucidate potential molecular mechanisms underlying this common arrhythmia. Functional properties of E428K, H445D, N470K, E655K, T1131I, R1826C and V1951M were assessed by whole-cell patch clamp recording of recombinant mutant channels heterologously expressed with the human β1 subunit in tsA201 cells. One variant (R1826C) did not exhibit substantial differences in biophysical properties of activation or fast inactivation, and another variant (E655K) only exhibited minor differences in recovery from inactivation as compared with wildtype (WT) channels. However, two mutants (H445D, T1131I) exhibited significant shifts in the voltage-dependence of activation toward more negative potentials ($p < 0.005$), and four other mutant channels (E428K, H445D, N470K, V1951M) exhibited significant shifts in the voltage-dependence of steady-state inactivation toward more positive potentials as compared with WT channels. Further, H445D and V1951M exhibited more rapid onset, impaired recovery and enhancement of slow inactivation evoked by 1000 ms depolarizing prepulses as compared with WT channels. For the five variants with either hyperpolarized activation voltage-dependence or depolarized steady-state inactivation, we predict increased window current as defined by the overlap of these two curves. Increased window current and enhanced slow inactivation of some variants is further predicted to alter excitability and/or conduction in myocardial tissue and is a plausible mechanism by which *SCN5A* variants may increase vulnerability to atrial fibrillation.