study is to estimate the contribution of the nNavs in the late sodium current ($I_{NaL}$) in ischemic condition in order to determine if an overexpression of the $nNa_vs$ can modify the APD and create an AS.
**Methods:** Freshly isolated cardiomyocytes were placed under ischemic conditions for 45 minutes. $I_{NaL}$ currents were recorded with the patch clamp technique in whole cell configuration. Tetrodotoxin (a specific $nNa_vs$ blocker) and MTSEA (a specific $Na_v1.5$ blocker) were used to differentiate the two sodium channels isoforms.
**Results:** In normal condition, $nNa_vs$ account for 11% of peak current. $I_{NaL}$ represents 0.3% of the peak current at a potential of −10 mV. Contribution of $nNa_vs$ (TTX sensitive) to $I_{NaL}$ was 36% ± 5%. Ischemia decreases the maximal current density from −73.3 nA/pF to −53.4 nA/pF. Surprisingly, $nNa_vs$ contribution was not modified (10% of the peak current). However, ischemia increases $I_{NaL}$ from 0.3% to 1.6% compared to the peak current.
**Conclusion:** Ischemia increases by 5.3 times $I_{NaL}$ that can play a critical role in the duration of the action potential and facilitates the outcome of arrhythmias.

**2688-Pos Board B458**
**Analysis of Voltage-Gated Sodium Channel Membrane Dynamics in Hippocampal Neurons via a Fluorescent Protein and Biotin Tagged Nav1.6 Channel**
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Voltage-gated sodium channels (Nav) are densely accumulated at the axon initial segment (AIS) of neurons where they are responsible for action potential initiation. The dense clustering of channels at the AIS involves ankyrinG binding, however the details of trafficking these channels to the AIS remains elusive. Furthermore, it is unclear what percentage of AIS channels is actually conducting. Since the large sodium channel cDNAs are difficult to manipulate and suffer from rearrangements in E. coli, the most elegant trafficking work to date has utilized chimeric proteins containing the sodium channel ankyrin-binding motif fused to other membrane proteins. To fully address trafficking in real time, an appropriately tagged full-length and functional channel is required. Therefore, we developed a Nav1.6 tagged with either GFP or Dendra2 fluorescent-proteins on the C-terminus and an extracellular biotin-acceptor-domain (BAD). The BAD allows for visualization and single molecule tracking of quantum-dot-bound sodium channels on the neuronal surface. This modified Nav1.6 demonstrated wild-type activity when expressed in hippocampal neurons. The tagged channel efficiently trafficked to the cell surface and was localized at the AIS as indicated by both confocal and TIRF microscopy. Alexafluor 594-conjugated-streptavidin binding indicated the surface-density of channels at the AIS was approximately 60 times greater than on the soma, comparable to endogenous Nav1.6 channels. Fluorescence recovery after photobleaching (FRAP) and single particle tracking showed that channels at the AIS had recovery time constants of greater than 2 hours and were confined to 60nm +/- 20nm compartments. In summary, we constructed a sodium channel with fluorescent protein and extracellular biotin reporters that has both wild-type trafficking and biophysical properties. This construct will permit the examination of sodium channel turnover, trafficking, diffusion and location-dependent function in neuronal cells.

**2689-Pos Board B459**
**NaV1.7 Splice Variant from Human Heart Compared with Neuronal hNaV1.7**
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Perception of noxious stimuli can be profoundly affected by mutations in the gene SCN9A which encodes the α-subunit of the voltage-gated sodium channel NaV1.7. Mutantions of this channel are associated with chronic pain or complete absence of pain. Primary erythermalgia and paroxysmal extreme pain disorder are syndromes associated with attacks of severe pain resulting from mutations that enhance NaV1.7 channel activity. Non-sense mutations in SCN9A lead to complete loss of NaV1.7 function. Loss of NaV1.7 function produces complete insensitivity to pain and anosmia, but little other changes in functions or behaviors. The pain-specific nature of the mutant NaV1.7 phenotypes is in keeping with the notion that this channel is expressed primarily in dorsal root ganglia and, to a lesser extent in the sympathetic ganglia. These premises make NaV1.7 an ideal target for the development of novel non-addictive analgesics. However, expression of NaV1.7 has been detected in the heart, with 5- to 10-fold higher levels in human cardiac Purkinje fibres versus the right atrium and ventricle and bradycardia and cardiac asystole have been reported in patients with paroxysmal extreme pain disorder. While these events have usually been ascribed to autonomic effects of the NaV1.7 mutations, a more direct effect of altered NaV1.7 in the heart cannot be ruled out. We have set out to clone and characterize the specific NaV1.7 subtype expressed in the human heart. In the present study we cloned and characterized a NaV1.7 subtype predominantly expressed in the human heart. This novel splice variant, missing one exon, may impact drug safety for this emerging family of analgesics. Its biophysical and pharmacological properties have been studied and will be discussed in comparison to the neuronal variant.

**2690-Pos Board B460**
**Biophysical and Pharmacological Characterisation of Native Human $Na_V1.8$ Channels from Isolated Dorsal Root Ganglia (DRG)**
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$Na_V1.8$ is a tetrodotoxin resistant (TTX-r) sodium channel expressed in sensory neurones that has a depolarised activation threshold, slow inactivation kinetics and a recovers rapidly from inactivation compared to tetrodotoxin sensitive (TTX-s) channels (Cummins & Waxman 1997; Akopian *et al.,* 1996). These biophysical properties mean that $Na_V1.8$ contributes to both electrogenesis and the maintenance of repetitive firing of action potentials (Blair & Bean 2002; Renganathan *et al.,* 2001; Waxman *et al.,* 2001). The expression and biophysical properties of $Na_V1.8$ can be modulated by ongoing nociceptive input and findings in the literature strongly support a key role for $Na_V1.8$ in pain signalling (England *et al.,* 1996; Roza *et al.,* 2003; Kerr *et al.,* 2001; Coward *et al.,* 2000; Akopian *et al.,* 1999). The selective $Na_V1.8$ antagonist A803467 has provided further evidence for a role of $Na_V1.8$ in nociceptive sensory input (Jarvis *et al.,* 2007) however characterisation and pharmacology of native $Na_V1.8$ currents has been shown using cells isolated from non human species. We have characterised $Na_V1.8$ currents recorded from TTX-r channels in human DRG and shown that they can be inhibited by a selective modulator of TTX-r current, A803467. Isolated currents had a $V_{1/2}$ inactivation of −35 mV and a $V_{1/2}$ of activation of −10 mV. Action potentials evoked by increasing current injections were inhibited by TTX and by the selective $Na_V1.8$ modulator A803467 which abolished repetitive firing. Furthermore, we have compared the biophysical properties of the native channel with those of recombinantly expressed human $Na_V1.8$ channel. This work not only provides a means by which we can assess the biophysics and pharmacological modulation of native human $Na_V1.8$ currents, but will also help us to understand the role of the channel in human pain signalling.

**2691-Pos Board B461**
**Input Signal Patterns Dominate a Plasticity of Spike-Onset Location at Cortical Pyramid Neurons through Local VGSCs**
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Synaptic signals drive the neurons to fire sequential spikes as digital codes. Short-term pulses initiate a spike at the axonal hillock, and physiological signals may initiate digital spikes at the soma. The regulation, mechanism and impact for spike-onset relocation between subcellular compartments remain unknown, which we investigated by simultaneously recording the soma and axon of pyramidal neurons in cortical slices. By analyzing the abilities of firing spikes, the time phases of spike-onset and the relocations of spike-initiation at these two compartments, we have found that long-time steady depolarization induces sequential spikes at the soma, but fluctuated one induces spikes at the axon. The soma in response to long-time pulses shows low thresholds and short refractory periods, or vice versa. Compared with axonal voltage-gated sodium channels (VGSC), somatic VGSCs in response to a pre-depolarization appear less inactivated and easily reactivated. Based on these VGSC features, the location of spike-onset simulated in *NEURON* model is consistent with the experiments. The patterns of input signals dominate spike initiations at the axon or soma of cortical pyramidal neurons through influencing local VGSC function. The plasticity of spike-onset location allows the neurons to program the brain codes economically. [This study is supported by the National Award for Outstanding Young Scientist (30325021), National Basic Research Program (2011CB504405) and Natural Science Foundation China (30870517, 30990261 and 81171033) to JHW].

**2692-Pos Board B462**
**A Markov Model for the Human Cardiac Sodium Channel**
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Voltage-gated sodium channels play an important role in the function of the human heart. Different voltage-clamp protocols were employed to determine kinetic and steady state voltage dependences that characterize channel gating. They include activation, deactivation, inactivation, and recovery from inactivation kinetics, current-voltage relationships, steady-state inactivation, and voltage dependence of normalized channel conductance ($G/G_{max}$). Several attempts were made to develop comprehensive mathematical model for sodium channel, however, most of them have noticeable limitations. We developed