that cannabidiol (CBD), the primary non-psychoactive constituent of *Cannabis sativa*, may protect against inflammation-induced neuropathy through its direct effects on neurological Nav1.6. To test these ideas, we used whole-cell patch clamp of human embryonic kidney (HEK 293) cells transiently transfected with SCN8A cDNA encoding neurological Nav1.6 α-subunit and incubated the cells under control conditions or in a cocktail of inflammatory mediators for 24 hours. Incubation in inflammatory mediators right-shifted the voltage dependence of both activation and steady-state fast inactivation, and increased late sodium current. Perfusion of CBD ($IC_{50}$; 5 μM) during recording rescued all the gating changes in Nav1.6 provoked by incubation with inflammatory mediators. These findings suggest that inflammation, by affecting Nav, is a potential pathogenic trigger for neurological pathologies. Moreover, CBD, through its direct effects on Nav, could potentially mitigate the dysfunction induced by inflammation.

**492-Pos**

**Characterization of atrial fibrillation linked mutations using the $Na_v1.5$ knock-out of atrial cardiomyocytes derived from iPSCs**

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Atrial fibrillation (AF) is the most common cardiac arrhythmia diagnosed and affects more than 33 million on people word wide. AF increases the risk of heart failure and myocardial infarction. Some studies carried out on HEK293 cells and murine models have shown that *SCN5A* mutations are linked to AF. However, the mechanism by which $Na_V1.5$ mutations generate cardiac arrhythmias is still to be elucidated. Human induced pluripotent stem cells (hiPSCs) hold great promise to model $Na_V1.5$-linked AF mutations and recapitulate the functional changes. Previously, our laboratory established an $Na_V1.5$ knock-out (KO) iPSC line with CRISPR/Cas9 technology. In this study, atrial cardiomyocytes (iPSC-aCMs) were differentiated from the Control and $Na_V1.5$ KO iPSC lines. We aim to characterise the atrial statue of the iPSC-aCMs, and then, use the model to express two AF Nav1.5-linked mutations (K1493R and M1875T). To verify that $Na_V1.5$ protein expression was suppressed, immunocytofluorescence and Western blot analysis were performed. Action potentials (APs) recordings in current-clamp mode showed that APs from the $Na_V1.5$ KO iPSC-aCMs exhibited a significant decrease of the AP overshoot and the upstroke velocity. Voltage-clamp analysis in $Na_V1.5$/K1493R-transfected KO iPSC-aCMs showed a shift of the steady-state activation toward hyperpolarized voltage while the $Na_V1.5$/M1875T expression provoked an increase of the $Na^+$ current density and a shift of the steady-state inactivation toward depolarized voltage. These alterations increase the window current which indicate a channel gain-of-function. The AP recordings showed a decrease of the depolarization threshold corresponding to a cellular excitability increase. 61 and 80 % of $Na_V1.5$/K1493R and /M1875T-transfected iPSC-aCMs respectively, exhibited arrhythmic events such as early and delayed afterdepolarizations (EADs and DADs). Our results confirm that the gain-of-function by shifting of the activation and inactivation properties is the cause of AF.

**493-Pos**

**Enhancement of EFL-inactivation gate interaction by truncation of Nav1.5 carboxy-terminal domain**

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The voltage-gated sodium channel 1.5 ($Na_V1.5$) participates in the initiation of the cardiac action potential by undergoing rapid activation which, within milliseconds, is followed by inactivation. Human channelopathic mutations in $Na_V1.5$ are linked to various cardiac maladies, including cardiac arrythmia, atrial fibrillation, and dilated cardiomyopathies. The carboxy-terminal domain (CTD) of $Na_V1.5$ is a well-recognized hotspot for channelopathic mutations. This domain harbors an EF-hand-like domain (EFL) and an IQ motif, which tune channel function via binding of auxiliary proteins, including the fibroblast growth factor homologous factors and calmodulin. In addition, the isolated $Na_V1.5$ EFL has been reported to bind the channel inactivation gate (IG) via a partially buried hydrophobic pocket. However, whether this binding occurs in the full-length human channel and how it tunes $Na_V1.5$ function are not well understood. Here, we have used high-resolution multi-channel electrophysiology recordings of disease-associated truncations and engineered $Na_V1.5$ mutants to probe this interaction. We show that truncation of the $Na_V1.5$ CTD increases late Na current, with a maximal increase observed for a truncation that includes helix V of the EFL ($Na_V1.5E[1864]*$), while further truncation resulted in reduced late Na current. Deletion of helix V increases the exposure of the EFL hydrophobic pocket, suggesting the large late Na current observed for $Na_V1.5E[1864]*$ may stem from a tighter interaction between the IG and the mutant EFL. Consistent with this, co-expression of $Na_V1.5E[1864]*$ with the WT $Na_V1.5$ CTD or IG reduced late Na current, while expression with IG mutants designed to disrupt binding with either the pore-domain (IFM/QQQ) or EFL (K1504E/K1505E) showed only a partial reduction. These findings suggest that as $Na_V1.5$ cycles between its functional states, the IG shuttles between the EFL and the pore domain shedding new light onto how these regions tune channel function.

**494-Pos**

**Effects of mexiletine on a race-specific mutation in Nav1.5 associated with long QT syndrome**

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Mutations in cardiac Nav1.5 have been associated with LQT syndrome, Brugada syndrome, and sudden arrhythmia death syndrome. Genetic studies showed that Nav1.5 mutations vary across race-ethnic groups. The Nav1.5 mutation P1090L was linked with familial long QT syndrome (LQTS) and was identified as an ethnic-specific variant in Asians. To explore the role of the P1090L in the LQT syndrome, we characterized the effects of the mutation on the Nav1.5 channel function. We found that the P1090L mutation altered the gating process of the channel and exhibited an enhanced window current. Treatment with mexiletine reversed the depolarization shift of the steady-state inactivation produced by the mutation. Mexiletine modified the recovery and development of inactivation of the mutation and reduced the mutated channel's availability. We characterized that the P1090L is a gain-of-function, the mutation presented a larger window current and increased channel availability. Treatment with mexiletine rescued the dysfunctional inactivation of the mutation. The results might potentially enable a mechanism-based approach to the treatment of LQTS.

**495-Pos**

**Elucidating the differential mutational effects of gating charges in voltage-gated sodium channels**

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Recent advances in genetics and pathophysiology have identified a large number of mutations in voltage-gated sodium ($Na_V$) channels that cause various heart, muscle, and brain excitability disorders. A significant feature of mutation distribution is that the disease-associated mutations are enriched in gating charges in voltage-sensing domains (VSDs). Intriguingly, the same mutation at the equivalent positions in different VSDs brings distinctive phenotypes.To address this mechanistic mystery, we studied the mutational effects on structural transitions of two gating charges (R2 and R3) in both VSD1 and VSD2 using molecular dynamics simulation and conformational free energy calculation. The application of an external electric field allows us to simulate the VSD transitions between ''up'' and ''down'' conformations at a microsecond scale. Our computational study shows that the structural impact of the same mutation (R to Q substitution) not only alters between different gating charges within the same VSD but also differs at the equivalent position between VSD1 and VSD2. The differential impacts are determined by a salt-bridge network. This network formed by gating charges and countercharges differs from one VSD to another. Our salt-bridge analysis reveals that the perturbations induced by mutations on this VSD-specific network bring unique impacts to the formation and stability of the intermediate state. The salt bridges in the intermediate play an unexpected role in determining the transition rate of VSDs, which elucidates a general principle behind the diverse mutational effects and will help us to predict the effects of more disease-associated mutations in voltage-gated ion channels.

**496-Pos**

**Effects of electric-field reshaping on voltage sensing in voltage-gated sodium channels**

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Voltage-gated sodium channels (Navs) are responsible for the depolarizing phase of the action potential with essential roles in fast electrical signalling. Membrane depolarization triggers opening of Nav channels via activation

of their voltage-sensing domains (VSD). The electric field across the membrane acts on a series of positively charged residues and induces their transmembrane relocation, which in turn triggers conformational changes that eventually lead to the opening of the sodium-conducting pore. Voltage dependence of the activation process can be described in terms of the gating charge that links energetics of VSD activation to the transmembrane voltage. Notably, the gating charge is defined by coupling of charged residues to the external electric field, whose shape is therefore crucial for Nav activation. Here, we employed molecular dynamics simulations of cardiac Nav1.5 and bacterial NavAb to gain atomic-level insights into the voltage-sensing mechanisms of Navs. Using our recently developed tool *g_elpot* to quantify VSD electrostatics with high spatial resolution, we found that, in contrast to earlier low-resolution studies, the electric field within VSDs of Nav channels has a complex isoform- and domain-specific shape, which prominently depends on the activation state of a VSD. Due to this field reshaping, not only translocated basic but also relatively static acidic residues contribute significantly to the gating charge. In the case of NavAb, we found that the transition between the resolved activated- and resting-state structures results in the gating charge of 8*e*, which is noticeably lower than experimental values of 12-16*e*. Our analysis of VSD electrostatics thus indicates that the resting-state structure represents an intermediate state of channel activation. In conclusion, our results provide an atomic-level description of the gating charge in Nav channels, and reveal the importance of electric-field reshaping for the energetics of voltage gating.

**497-Pos**

**High-level expression of voltage-gated sodium channels in thymidine-arrested human embryonic kidney cells**

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The benefit of using heterologous mammalian cell lines such as human embryonic kidney (HEK293) cells for expressing voltage-gated ion channels includes efficient translation and processing of such large membrane proteins. Still, the biophysical assessment of these channels is hindered by their low expression and reduced localization on the surface of a cell. Having a higher expression level of ion channels is crucial for investigating mutations that cause a decrease in the current amplitude as well as for measuring tiny currents such as gating currents and gating pore currents that are in the range of 0.1 to 1% of the peak current. We aimed at improving the efficiency of ion channel expression and enhancing their localization in cell membranes by modifying the protocol of cell cycle arrest at the G1/S boundary using thymidine to direct the cells into increasing the expression machinery during viral transduction of mammalian voltage-gated sodium channels. The combination of cell arrest at the G1/S boundary and mammalian baculovirus system (BacMam) resulted in a 5-fold increase in the current density of different mammalian voltage-gated sodium channels in HEK293 cells compared to the current literature. By applying this modified protocol for expression, we succeeded to measure a pathogenic gating pore current in HEK293 cells of ∼0.5% of the central pore current induced by an autism-related mutation in the R2 gating charge in Domain II (R853Q) of $Na_v1.2$, which is blocked in a voltage-dependent manner when the voltage sensors activate. These results show that our protocol is a universal one that can be applied on different voltage-gated ion channels and possibly other membrane proteins. This ultimately will pave the way to decipher the structure and function of ion channels and their association with ion channelopathies and other diseases.

**498-Pos**

**A novel high throughput combined voltage-clamp/current-clamp analysis of single primary neurons**

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The patch-clamp technique is the gold-standard methodology for biophysical investigations of channels and receptors studied by voltage-clamp, and for analysis of excitability of cells, such as neurons studied via current-clamp. However, the throughput of manual patch-clamp is slow, and high-throughput robotic patch-clamp, while helpful for assessments, such as drug screening, has been primarily used to study channels and receptors expressed in heterologous expression systems. In this study, we introduce a novel approach to automated high throughput patch-clamping that substantially enhances high throughput analysis of excitable cells at the channel and cellular level. As a proof-of-concept, we apply this approach to investigate the detailed biophysical properties of voltage-gated sodium (Nav) channels in dorsal root ganglion (DRG) neurons, which are among the most diverse and complex neuronal cells. Our approach enables high throughput, unbiased, fast, simultaneous, and head-to-head electrophysiological recordings from a wide range of primary neurons. Furthermore, our approach eliminates the need for culturing of cells on coverslips and provides the ability to perform both voltage- and current-clamp recordings on the same neuron. This approach can be used for many applications, including both physiological and pharmacological analyses of primary DRG neurons.

**499-Pos**

**Fine-structure constant and hysteresis regimes in the giant squid propagating action potential**

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We present a phase space based phenomenological theory for the steady propagation of the action potential using the Rosenthal-Bezanilla experimental data. Time derivatives of giant squid action potential, its velocity of propagation, the radius of the axon and its capacitance, and the resistivity of axoplasm, yield the total ionic, capacitive, and membrane currents of the charge conserving cable equation. Evidence is presented that the sodium channels lattice has a polarization flip at the inception of the action potential and the corresponding flip reversal at the peak of the action potential while traversing a ferroelectric hysteresis loop. The polarization flip at the inception of the action potential is followed by capacitive polarization current known as gating current. The polarization flip at the peak of the action potential is preceded by a small sodium polarization current associated with sodium current inactivation. Ionic currents and sodium polarization ionic currentsare taken to have the familiar structure, as the product of maximum conductance, driving force, and fraction of open channels. Fractions of open channels are fitted in the lab frame by modified Avrami equations seeded with the value of the fine-structure constant $\alpha = 0.0072973...$ . The existence of sodium channels domains with two different symmetries, albeit only one being stable in squid axon, suggests the possibility of neurons with at least 2 stable states, the necessary condition for storage and retrieval of memories. It is expected that presented results will provide the framework for further analysis of thermodynamic phase changing behavior, the role of quantum mechanics in the flow of ions trough ionic channels and in particular the role of ferroelectric sodium channels lattice behavior in storage and retrieval of memories and nerve excitability.

**500-Pos**

**No evidence of functional interactions between cardiac sodium channels**

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Cardiac voltage gated sodium ($Na^+$) channels ($Na_v1.5$) are central for myocardial excitation. Based on single-channel recordings, it was recently proposed that $Na^+$ channels interact and exhibit coupled gating. However, single-channel recordings are typically processed manually, which is a biased procedure. To allow an unbiased assessment of possible channel interactions, we developed an automated pipeline to detrend and idealize single-channel currents.

Cell-attached experiments were performed in HEK293 cells expressing wild-type human $Na_v1.5$ channels (n=11) and adult mouse ventricular cardiomyocytes (n=4). Individual sweeps were detrended by linear optimization using a library of exponential functions, digitally filtered and the baseline was offset. The novel idea behind the next step is that the average of the idealized currents identified by thresholds between current levels reconstructs at best the ensemble average current. The thresholds were set at the midpoints between current levels and the mean square residual with respect to the ensemble average current was computed. The thresholds were then adjusted automatically using nonlinear optimization to minimize this residual. This procedure was repeated under the assumption of different numbers of channels. The smallest residual was reached when this number corresponded to the number of channels. Thus, counting open channels at any given time was unbiased. To ascertain interactions between channels, the distribution of these counts was compared statistically to that expected for independent channels, and the difference in Shannon's entropy between these distributions was computed. In all the investigated patches so far, our analysis indicates that the interaction between $Na^+$ channels is not significant, in contrast to what was reported by others.