A Selective $Na_V1.1$ Potentiator Enhances Interneuron Excitability to Normalize Motor Performance in a Dravet Syndrome Mouse Model

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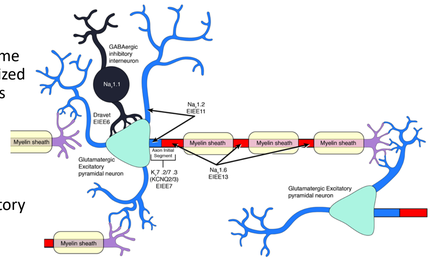
INTRODUCTION

- Loss-of-function variants of SCN1A cause Dravet Syndrome (SMEI or EIEE6) and generalized epilepsy with febrile seizures plus (GEFS+), by decreasing $Na_V1.1$ expression or conductance in inhibitory interneurons. The resulting hypo-excitability of interneurons reduces inhibitory input on excitatory neurons and leads to epilepsy and developmental delays



- A precision medicine therapy for Dravet Syndrome should restore $Na_V1.1$ activity specifically without impacting other neuronal proteins, especially ion channels
- We are pursuing brain penetrant small molecule potentiators of $Na_V1.1$ channels to allow oral dosing and modulation of the $Na_V1.1$ current levels in all brain areas
- We believe that such potentiators can directly address the underlying etiology of Dravet Syndrome and thus provide a potentially disease modifying therapy for Dravet Syndrome

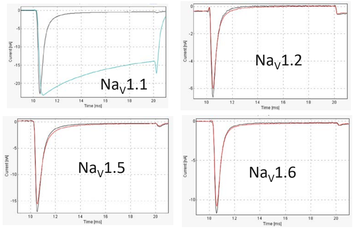
METHODS

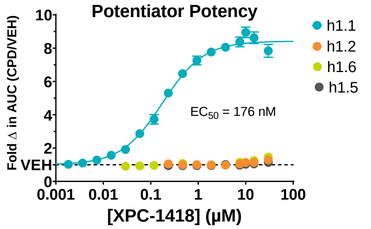
- **Voltage clamp electrophysiology** was used to assess the potency and selectivity of compounds in HEK cell lines stably expressing Na_V's on the Sophion Qube-384. Potency was measured by determining the increase in charge carried over 10 ms. Availability curves were generated by assessing current at test pulse following 500 ms prepulses to -120 to 0 mV in 10 mV steps. Error bars are ±SEM
- **Electrophysiological Recordings in Brain Slices**. *Scn1a*$^{+/-}$ mice and wildtype (WT) littermates were generated as described previously[1]. Postnatal day (P0-3) mice were injected (i.c.v) with AAV-E2-Tdtomato virus. 400 µm parasagittal cortical brain slices were prepared from P21-28 mice using standard procedures[2] Whole-cell current-clamp recordings were made in cortical layer 5. Fast-spiking interneurons expressing viral reporter were targeted for patching. sIPSCs and sEPSCs were recorded from layer 5 pyramidal cells in presence of NBQX/AP5 and Gabazine at HP of 20 mV and -70 mV respectively in voltage-clamp. Error bars are ±SEM
- ***Scn1a*$^{+/-}$ 6 Hz seizure model**. Seizures were induced in 20-22 days-old *Scn1a*$^{+/-}$ male mice by a 6 Hz stimulus for 3 seconds delivered through corneal electrodes and the CC97 was determined. Mice were stimulated at this current and placed in a plexiglass chamber to monitor for the presence of a seizure characterized by jaw clonus, forelimb clonus, Straub tail and loss of balance. An animal was considered "protected" if none of these 4 behaviors occurred. A mouse is considered seizing if at least one of these behaviors was observed. Binary seizure data were assessed with simple logistic regression to determine the concentration for 50% probability of protection ($X_{50\%}$). $EC_{min,b,u}$ is calculated as the mean free concentration of the 3 protected mice with the lowest brain exposures
- **Rotarod.** *Scn1a*$^{+/-}$ male mice were tested at P25-28. Mice were placed on an accelerating rotating rod (acceleration from 2 to 15 RPM over 3 min) for habituation. An hour later mice were placed on an accelerating rod (2 to 30 RPM over 3 min) and baseline latency recorded. An hour later mice were administered with the treatment. An hour later, the test was performed and repeated 3 times for each mouse and average latency is reported

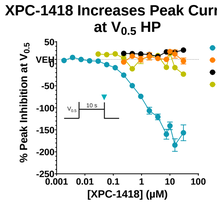
RESULTS

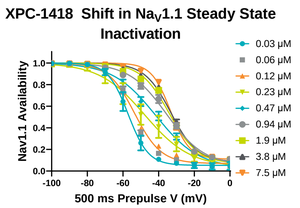
Potency, Selectivity and Mechanism of Action (MOA) of XPC-1418

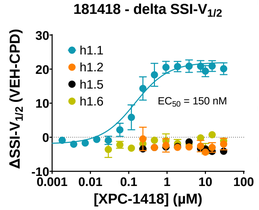
- XPC-1418 is a representative lead compound that stabilizes the open state of the $Na_V1.1$ channel selectively with an EC_{50} of 176 nM
- Traces shown with 10 µM of XPC-1418 demonstrate the high selectivity for human $Na_V1.1$ channels and sparing of human neuronal channels $Na_V1.2$ and $Na_V1.6$ and cardiac channel $Na_V1.5$
- The potency is measured from the area under the current (AUC) trace during a step depolarization to 0 mV for 10 ms from a holding potential of -120 mV







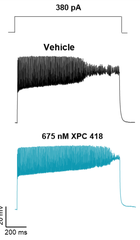


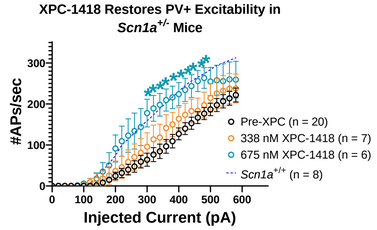


- XPC-1418 destabilizes steady state inactivation (SSI) and increase channel availability across a range of potentials close to neuronal resting membrane potentials
- No effects on the voltage dependence of activation are observed (not shown)
- Potency determined by the shift in SSI EC_{50} = 150 nM, similar to the AUC EC_{50}

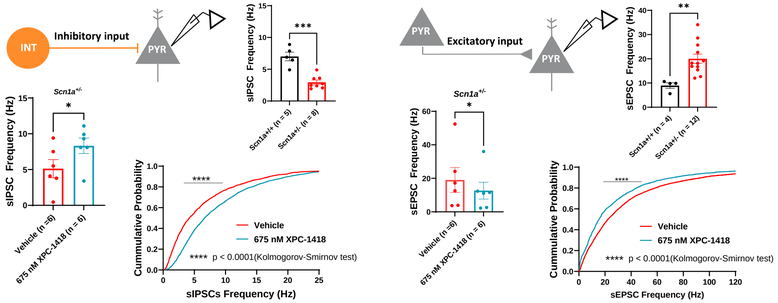
XPC-1418 Selectively Increases Firing Rate of *Scn1a*$^{+/-}$ Inhibitory Neurons

- In brain slices from *Scn1a*$^{+/-}$ mice, PV+ interneuron firing frequency was significantly increased by XPC-1418 indicating a higher firing frequency of fast spiking inhibitory interneurons (*P*<0.05, 2-way ANOVA)
- No significant effects were seen when XPC-1418 was applied to WT inhibitory PV+ interneurons at similar concentrations (not shown)

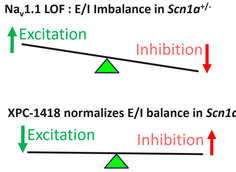




XPC-1418 Normalizes Spontaneous Post Synaptic Inhibitory and Excitatory Currents in *Scn1a*$^{+/-}$ Neurons

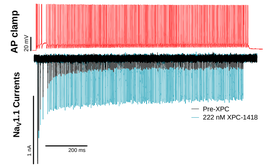


- *Scn1a*$^{+/-}$ mice display lower sIPSC frequency and higher sEPSC activity than WT (unpaired t-tests)
- XPC-1418 significantly increases sIPSC frequency and reduces sEPSC activity toward WT levels (unpaired t-test)

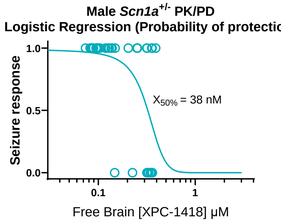


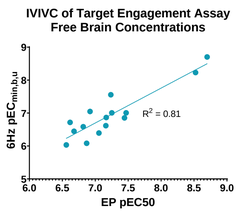
Action Potential Clamp Demonstrates Suppression of $Na_V1.1$ Channel Inactivation During High Frequency Activity

- Applying an action potential voltage clamp command from PV+ neuron current clamp recordings to $Na_V1.1$ expressing cells shows XPC-1418 slows the accumulation of inactivation of $Na_V1.1$ channels which could lead to reduced neuronal excitability through AP failure and depolarization block



$Na_V1.1$ Potentiators Suppress 6 Hz Seizures in Target Engagement Assay

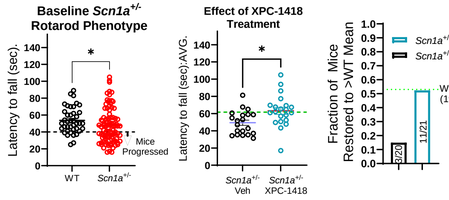




- *Scn1a*$^{+/-}$ 6 Hz seizure assay evokes seizures only in *Scn1a*$^{+/-}$ animals and confirms target engagement. Efficacy is achieved at an approximate $Na_V1.1$ receptor occupancy of 20%
- Strong In vitro in vivo correlation (IVIVC) across a selection of compounds between electrophysiological potency and in vivo 6Hz confirms that efficacy is driven by target engagement

XPC-1418 Restores Motor Performance in *Scn1a*$^{+/-}$ Mice

- Significantly improved performance on the Rotarod assay of *Scn1a*$^{+/-}$ mice suggests efficacy of XPC-1418 against non-seizure related symptoms such as motor dysfunction (Unpaired t-test)



CONCLUSIONS

- XPC-1418 is a CNS penetrant, highly $Na_V1.1$ selective small molecule potentiator that stabilizes open states, increases channel availability and increases Na^+ flux upon depolarizing inputs
- This MOA increases impaired *Scn1a*$^{+/-}$ interneuron excitability and normalizes excitation/inhibition imbalance in *Scn1a*$^{+/-}$ mice
- XPC-1418 demonstrates target engagement in vivo by preventing seizures in a *Scn1a*$^{+/-}$ 6 Hz target engagement seizure model and improved Rotarod performance supporting the potential efficacy of this mechanism in non-seizure related symptoms
- XPC-1418 provides a new, mechanistically differentiated class of voltage-gated sodium channel compounds with the potential to provide an improved therapeutic profile for the overarching treatment of Dravet Syndrome

FUNDING Xenon Pharmaceuticals Inc.

DISCLOSURES Samuel J. Goodchild, Kristen Burford, Celine Dube, Samrat Thouta, Esteban Suarez Delgado, Ryley Parrish, Aaron D. Williams, Alison Cutts, Maegan Soriano, Richard Dean, Verner Lofstrand, Helen Clement, Davie Kim, Steven Wesolowski, James Empfield, and J.P. Johnson Jr. are employees of and own stock or stock options in Xenon Pharmaceuticals Inc.

REFERENCES **1.** Miller AR, Hawkins NA, McCollom CE, Kearney JA. *Genes Brain Behav*. 2014;13(2):163-172. **2.** Tai C, Abe Y, Westenbroek RE, Scheuer T, Catterall WA. *Proc Natl Acad Sci U S A*. 2014;111(30):E3139-E3148.

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