Selective Potentiation of $Na_V1.1$ Channels in Dravet Mice Restores Interneuron Function and Improves Motor Function

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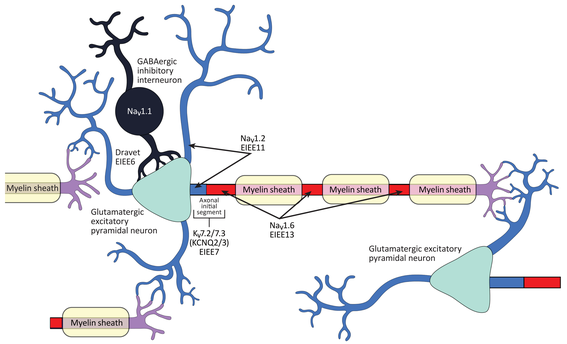
INTRODUCTION

- 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 hypoexcitability of interneurons reduces inhibitory input on excitatory neurons and leads to epilepsy and developmental delays[1,2]
- A precision medicine therapy for Dravet syndrome should restore $Na_V1.1$ activity specifically without impacting other neuronal proteins, especially ion channels
- We are developing orally available $Na_V1.1$ potentiators that can directly target the underlying etiology of Dravet syndrome and thus provide a potentially disease modifying therapy for Dravet syndrome



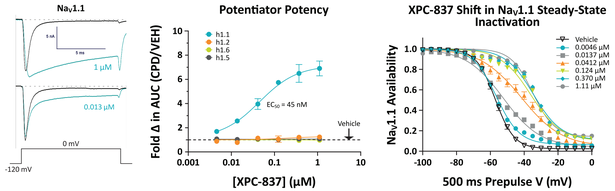
METHODS

- **Voltage clamp automated electrophysiology** was used to assess the potency and selectivity of compounds in HEK cell lines stably expressing Na_V's. Error bars are ± SEM
- **Electrophysiological recordings in brain slices**. 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
- ***Scn1a*$^{+/-}$ 6 Hz and rotarod**. *Scn1a*$^{+/-}$ male mice were tested at P21-22 and P24-P28, respectively
- ***Scn1a*$^{+/-}$ spontaneous seizure assay/SUDEP**. *Scn1a*$^{+/-}$ mice were fed with medicated chow from P21-36
- **Long-term potentiation (LTP) hippocampal local field potentials (LFPs)** were recorded from sagittal brain slices from female mice administered with medicated chow for 14 days. Stimulation of the CA3 schaffer collaterals with a theta-burst high-frequency stimulation induced LTP in CA1 hippocampal neurons and compared between groups (1-way ANOVA)

RESULTS

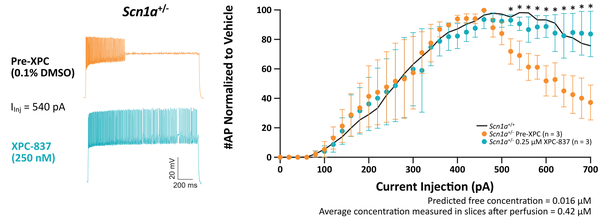
XPC-837 Potently and Selectively Potentiates $Na_V1.1$

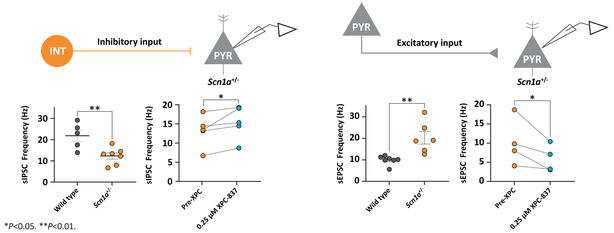
- XPC-837 stabilizes the open state of the $Na_V1.1$ channel selectively with an EC_{50} of 45 nM
- XPC-837 destabilizes steady-state inactivation (SSI) and increases channel availability across a range of potentials close to neuronal resting membrane potentials



XPC-837 Normalizes Interneuron Function in *Scn1a*$^{+/-}$ Mice

- In brain slices from *Scn1a*$^{+/-}$ mice, PV+ interneuron firing frequency was significantly increased by XPC-837 at current injections where depolarization block occurred, indicating a higher firing frequency of fast spiking inhibitory interneurons (P<0.05, 2-way ANOVA)
- *Scn1a*$^{+/-}$ mice display lower sIPSC frequency and higher sEPSC activity than wild type (unpaired t-tests)
- XPC-837 significantly increases sIPSC frequency and reduces sEPSC activity in *Scn1a*$^{+/-}$ towards wild type levels (paired t-test)

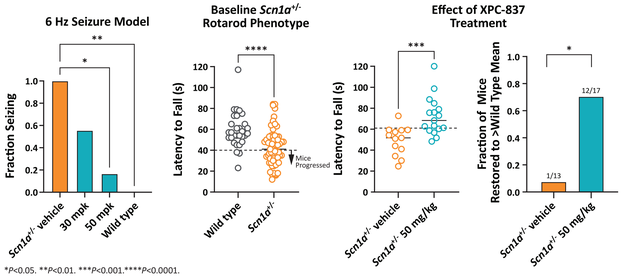




*P<0.05. **P<0.01.

A Single Oral Dose of XPC-837 Suppresses Induced Seizures and Improves Motor Performance in *Scn1a*$^{+/-}$ Mice

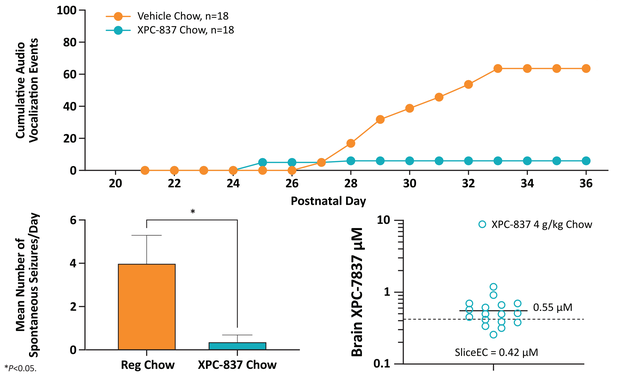
- *Scn1a*$^{+/-}$ 6 Hz seizure assay evokes seizures only in *Scn1a*$^{+/-}$ animals, and suppression of seizures with XPC-837 confirms target engagement
- XPC-837 rescues function in the rotarod assay of *Scn1a*$^{+/-}$ mice, suggesting efficacy against non-seizure–related symptoms such as motor dysfunction



*P<0.05. **P<0.01. ***P<0.001.****P<0.0001.

Chronic Oral Dosing of XPC-837 Protects *Scn1a*$^{+/-}$ Mice From Spontaneous Seizures

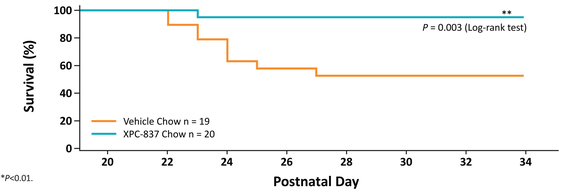
- Male *Scn1a*$^{+/-}$ mice were fed with chow containing 4 g/kg of XPC-837 or regular chow from P21 until P36
- Audio detection of vocalizations associated with behavioral seizure was used to identify spontaneous seizures that were confirmed with manual examination of video
- Chronic dosing led to significant suppression of seizures with brain exposures at the end of the experiment on P36 of 0.55 µM. The chronic brain exposure is similar to the ex vivo concentration found to be efficacious in brain slices to correct PV interneuron deficits in firing



*P<0.05.

Chronic Oral Dosing of XPC-837 Protects *Scn1a*$^{+/-}$ Mice From SUDEP

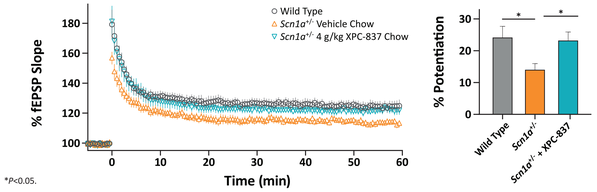
- Female *Scn1a*$^{+/-}$ mice were fed with chow containing 4 g/kg of XPC-837 or regular chow from P21 until P34 and cages were checked twice daily for SUDEP
- XPC-837 medicated chow significantly protected mice from SUDEP



**P<0.01.

Chronic Oral Dosing of XPC-837 in *Scn1a*$^{+/-}$ Mice Increases Long-Term Potentiation (LTP)— A Potential Cellular Correlate of Learning and Memory

- Female *Scn1a*$^{+/-}$ mice were fed chow containing 4 g/kg of XPC-837 or regular chow from P21 until P34 before LTP recordings were made



*P<0.05.

CONCLUSIONS

- XPC-837 is an orally available CNS penetrant, highly $Na_V1.1$ selective small molecule potentiator that stabilizes open states and increases channel availability and Na^+ flux
- This MOA increases *Scn1a*$^{+/-}$ interneuron excitability and normalizes excitation/inhibition imbalance in *Scn1a*$^{+/-}$ mouse neurons
- Acute dosing of XPC-837 suppresses induced seizures in the 6 Hz assay and improves motor performance in the rotarod assay, supporting the potential for improvements in Dravet patient motor function
- Chronic dosing over 14 days with XPC-837 in chow supresses spontaneous seizures, prevents SUDEP, and increases long-term potentiation
- XPC-837 represents a novel, mechanistically differentiated, orally available compound with the potential to provide an improved therapeutic profile for the overarching treatment of Dravet syndrome

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DISCLOSURES All authors are employees of and own stock or stock options in Xenon Pharmaceuticals Inc.

REFERENCES 1. Miller AR, et al. *Genes Brain Behav.* 2014;13(2):163-172. 2. Tai C, et al. *Proc Natl Acad Sci USA.* 2014;111(30):E3139-E3148.

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