Selective Potentiation of $Na_V1.1$ Channels by XPC-837 in Dravet Mice Suppresses Spontaneous Seizures, Prevents SUDEP and Increases LTP

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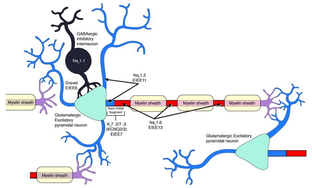
INTRODUCTION

- Loss-of-function variants of *SCN1A* cause Dravet Syndrome 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[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 small molecule $Na_V1.1$ potentiators that can directly target the underlying etiology of Dravet Syndrome and thus provide a potentially disease modifying therapy for Dravet Syndrome.

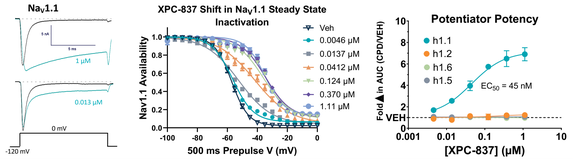
METHODS

- **Voltage clamp automated electrophysiology** was used to assess potency and selectivity of XPC-837 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.
- ***Scn1a*$^{+/-}$ Mouse** line was described in Miller et al.[3]
- ***Scn1a*$^{+/-}$ 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.
- **LTP** Hippocampal local field potentials were recorded from sagittal brain slices from female *Scn1a*$^{+/-}$ mice administered with medicated chow for 14 days. Stimulation of the CA3 Schaffer collaterals with a theta burst high frequency stimulation induced a long-term potentiation (LTP) in CA1 hippocampal neurons and compared between groups (one way ANOVA).
- **RNAseq:** Saphenous vein blood or hippocampal tissue was taken from wild type, *Scn1a*$^{+/-}$, and *Scn1a*$^{+/-}$ + XPC837 mice at P25-27 and P35, respectively. Mice and samples were individually identified in a spontaneous seizure study for downstream analysis. Significantly differential gene expression (DEG) patterns were identified; all DEGs shown have p<0.05 for a change between analyzed groups, when corrected for multiple testing using the Benjamini-Hochberg (BH) false discovery rate.

RESULTS

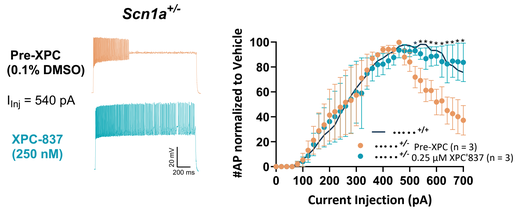
XPC-837 Potently and Selectively Potentiates $Na_V1.1$

- 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 and increases channel availability across a range of potentials close to neuronal resting membrane potentials.



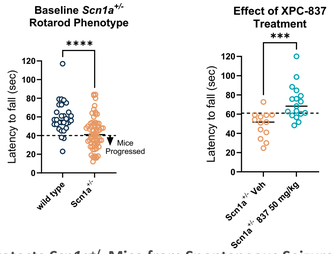
XPC-837 Normalizes Interneuron Function in *Scn1a*$^{+/-}$ mice

- 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.

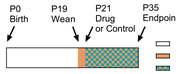


A Single Oral Dose of XPC-837 Improves Motor Performance in *Scn1a*$^{+/-}$ Mice

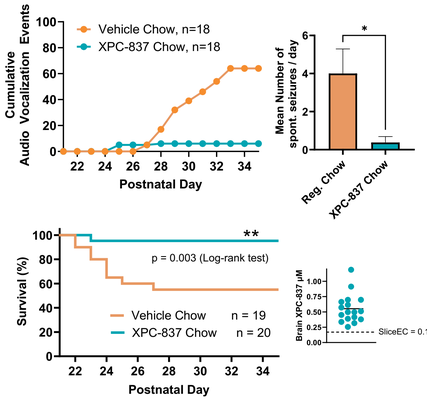
- XPC-837 rescues function in the Rotarod assay of *Scn1a*$^{+/-}$ mice suggesting efficacy against non-seizure related symptoms such as motor dysfunction.



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



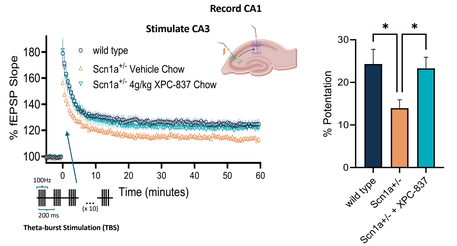
- *Scn1a*$^{+/-}$ mice were fed with chow containing 4g/kg of XPC-837 or regular chow from P21 until P35.
- Audio detection of vocalizations associated with behavioral seizure was used to identify spontaneous seizures in male mice that were confirmed with manual examination of video.
- Chronic dosing 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 slice to correct PV interneuron deficits in firing.
- Female *Scn1a*$^{+/-}$ mice were significantly protected from SUDEP.



Legend for all Figures: * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001

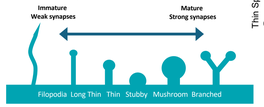
Chronic Oral Dosing of XPC-837 in *Scn1a*$^{+/-}$ Mice Increases Long Term Potentiation – A Potential Cellular Correlate of Learning and Memory

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

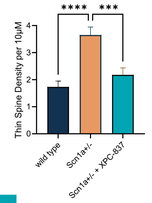


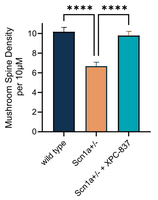
XPC-837 Produces a More Mature Spine Morphology in *Scn1a*$^{+/-}$ mice

- The deficit in female *Scn1a*$^{+/-}$ mouse dendritic spine maturation[5] is corrected by XPC-837.
- XPC-837 decreased thin spines and increased mushroom spines.



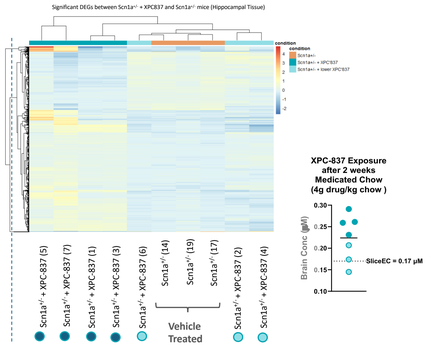
Adapted from Risher et al.[4]





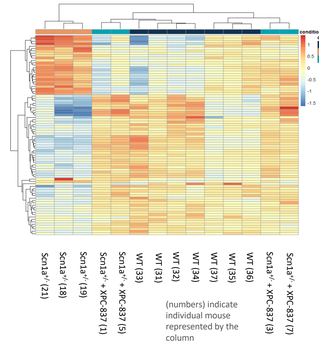
XPC-837 Modulates Hippocampal Gene Expression in *Scn1a*$^{+/-}$ mice

- XPC-837 Brain exposure in female *Scn1a*$^{+/-}$ mice at P35 correlates with significant differentially expressed gene (DEG) profiles in the hippocampus when compared to untreated *Scn1a*$^{+/-}$ mice.[6]
- Mice with lower XPC-837 brain exposure have DEG expression profiles similar to untreated *Scn1a*$^{+/-}$ mice.
- *Scn1a*$^{+/-}$ mouse #19 is a surviving untreated animal with seizures, #14 & #17 clustered around this 'symptomatic' *Scn1a*$^{+/-}$ mouse.



XPC-837 Modulates Peripheral Gene Expression in *Scn1a*$^{+/-}$ mice

- For genes that are significantly different in wild type and *Scn1a*$^{+/-}$ mice (DEGs), high exposure XPC-837 *Scn1a*$^{+/-}$ mice group with wild type mice.
- Untreated *Scn1a*$^{+/-}$ mice group together separately.
- Suggests that XPC-837 realigns *Scn1a*$^{+/-}$ 'disease-related' genes back toward wild-type levels.
- Blood sampling of mice early (P25-27) avoids survivor bias that can be present in the brain tissue data analysis at P35.



CONCLUSIONS

- XPC-837 is an orally available, CNS penetrant, highly $Na_V1.1$-selective small molecule potentiator that stabilizes open states, increases channel availability, and Na^+ flux.
- This MOA increases interneuron excitability in *Scn1a*$^{+/-}$ mouse neurons.
- Acute dosing of XPC-837 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, increases LTP, and produces a more mature dendritic spine morphology.
- RNAseq data demonstrates patterns of gene expression differences in blood cells between wild type and *Scn1a*$^{+/-}$ mice; these differences were normalized by administration of XPC-837 to *Scn1a*$^{+/-}$ mice.
- Efficacious concentrations of XPC-837 in chronic dosing experiments in *Scn1a*$^{+/-}$ mice were similar across assays and consistent with brain slice data.
- 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.

References **1.** Claes et al., Am J Hum Genet. 2001 May 15;68(6):1327–1332. **2.** Tai et al. *Proc Natl Acad Sci U S A.* 2014;111(30):E3139-E3148. **3.** Miller et al. *Genes Brain Behav.* 2014 Nov 14;13(2):163–172. **4.** Risher et al. *PLoS One.* 2014 Sep 10;9(9):e107591. **5.** Pizzamiglio et al. *Neurobiol Dis.* 2025 Apr:207:106853 **6.** Hawkins NA, et al. Exp Neurol. 2019 Jan:311:247-256.

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