Selective Potentiation of Inhibitory Networks Prevents Seizures in a Mouse Model of Dravet Syndrome

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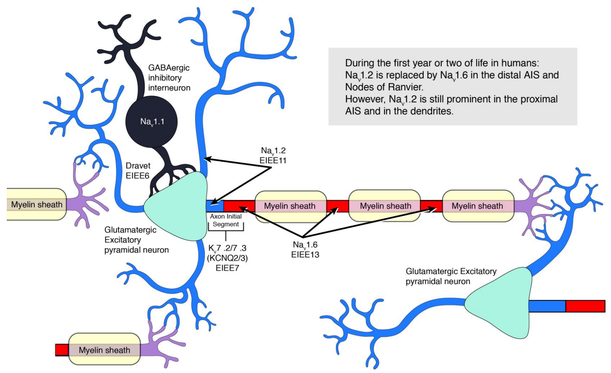
BACKGROUND

- 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 or conductances.
- We are pursuing brain penetrant small molecule enhancers of $Na_V1.1$ currents to allow oral dosing and titration of the $Na_V1.1$ current levels.
- We believe that such activators can directly address the underlying cause of Dravet Syndrome with the potential to provide a safe and effective pharmacotherapy.

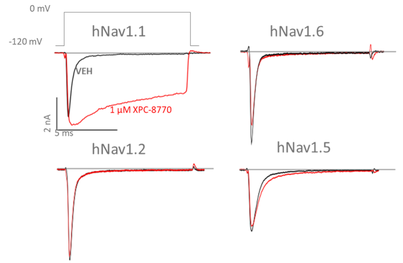


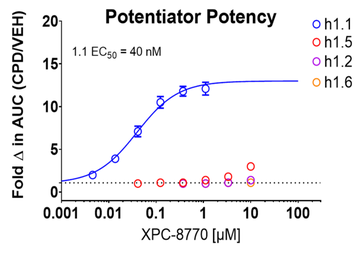
METHODS

- **Voltage clamp electrophysiology** was used to assess the potency and selectivity of XPC-8770 using the Sophion Qube-384. Potency was measured by determining the increase in charge carried over 10 ms.
- **Animals**. *Scn1a*$^{+/-}$ mice were generated as described previously.[1]
- **Brain Slice Preparation**. 400 µm parasagittal cortical brain slices were prepared from >P21 mice using standard procedures[2]
- **Electrophysiological Recordings in Brain Slices**. Whole-cell current-clamp recordings were made in cortical layer 5 (20-22°C). Fast-spiking interneurons were identified by their characteristic fast-spiking pattern and confirmed *post hoc* by single-cell RT-PCR.
- ***Scn1a*$^{+/-}$ 6 Hz seizure model**. Seizures were induced in 20-22 days-old *Scn1a*$^{+/-}$ 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.

RESULTS

Potency, Selectivity and Mechanism of Action (MOA) of XPC-8770

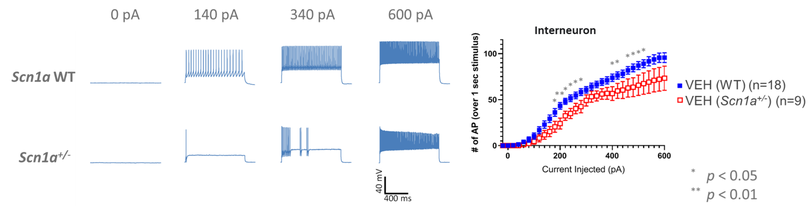




- XPC-8770 selectively potentiates $hNa_V1.1$ channels and spares neuronal channels $Na_V1.2$ and $Na_V1.6$ and cardiac channel $Na_V1.5$. XPC-8770 acts on $Na_V1.1$ by impairing inactivation of the channel.
- For subsequent neuronal experiments we used a saturating concentration of 1 µM to target the $Na_V1.1$ channels as well as a concentration of 150 nM to look for a concentration response of effect.

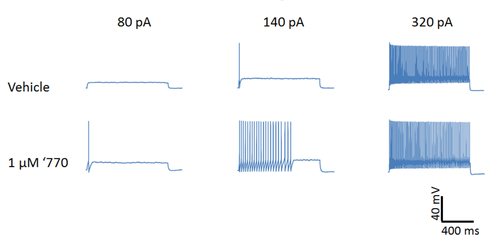
Compound	$Na_V1.1$ EC_{50} (µM)	$Na_V1.6$ EC_{50} (µM)	$Na_V1.2$ EC_{50} (µM)	$Na_V1.5$ EC_{50} (µM)	Selectivity $Na_V1.1/1.6$
Dominant Channel Expression	*CNS: Fast Spiking Inhibitory Interneurons*	*CNS: Excitatory Neurons*	*CNS: Excitatory Neurons*	*Heart: Cardiomyocytes*	
XPC-8770	0.040	>30	>30	>30	>750

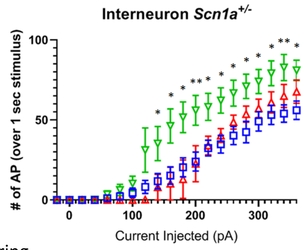
Shift in Rheobase and Decreased Maximal Firing Rate in *Scn1a*$^{+/-}$ vs. Wild Type (WT) Inhibitory Neurons



- When brain slices from wild-type mice and *Scn1a*$^{+/-}$ mice are compared, a shift in rheobase and decreased maximal firing rate in *Scn1a*$^{+/-}$ inhibitory neurons is observed.

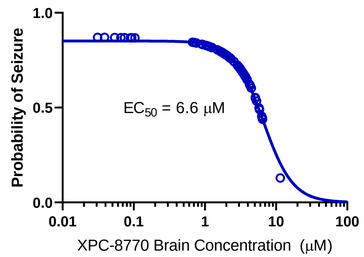
XPC-8770 Increases Firing of *Scn1a*$^{+/-}$ Inhibitory Neurons





- In brain slices from *Scn1a*$^{+/-}$ mice, XPC-8770 increased the firing rate of inhibitory interneurons at 1 µM but not 150 nM.
- No effects were seen when XPC-8770 was applied to WT inhibitory interneurons.
- XPC-8770 treatment improved interneuron excitability, increasing maximum firing rate and preventing collapse of firing at high stimulus input.

XPC-8770 Suppresses Seizures in a *Scn1a*$^{+/-}$ Mouse 6 Hz Seizure Model



- *Scn1a*$^{+/-}$ 6 Hz seizure assay evokes seizures specifically in *Scn1a*$^{+/-}$ animals but not WT animals.
- XPC-8770 reduced the probability of *Scn1a*$^{+/-}$ mice seizing with an EC_{50} of 6.6 µM.

CONCLUSIONS

- XPC-8770 is a highly selective small molecule potentiator of $Na_V1.1$.
- Compound binding impairs fast inactivation and increases Na^+ flux and cellular excitability.
- Selectively potentiating $Na_V1.1$, the dominant sodium channel isoform expressed in inhibitory interneurons, restores the capability of mouse *Scn1a*$^{+/-}$ interneurons to fire action potentials at high frequency.
- The compound showed efficacy in a *Scn1a*$^{+/-}$ 6 Hz seizure model, providing *in vivo* proof of concept for this mechanism of action.
- This profile provides a new, mechanistically differentiated, class of voltage-gated sodium channel potentiators with the potential to provide an improved therapeutic profile for the treatment of Dravet Syndrome.

[1] Miller AR, Hawkins NA, McCollom CE, Kearney JA. Mapping genetic modifiers of survival in a mouse model of Dravet syndrome. *Genes Brain Behav*. 2014;13(2):163–172. doi:10.1111/gbb.12099

[2] Tai C, Abe Y, Westenbroek RE, Scheuer T, Catterall WA. Impaired excitability of somatostatin- and parvalbumin-expressing cortical interneurons in a mouse model of Dravet syndrome. Proc Natl Acad Sci U S A. 2014;111(30):E3139–E3148. doi:10.1073/pnas.1411131111

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