Small Molecule Potentiators of $Na_V1.1$ Increase Action Potential Firing in Fast Spiking Cortical Inhibitory Interneurons from a Mouse Model of Dravet Syndrome

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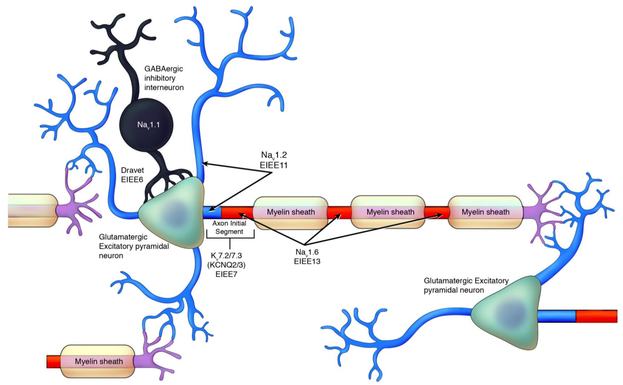
BACKGROUND

- 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 hope that such activators can directly address the underlying cause of Dravet Syndrome with the potential to provide a safe and effective pharmacotherapy.

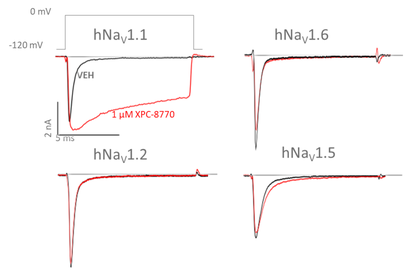


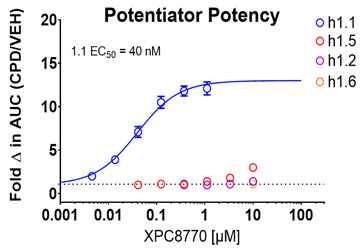
METHODS

- **Patch clamp electrophysiology** with HEK cells expressing $Na_V1.x$ was used to examine the potency and selectivity of XPC-8770 using the Sophion Qube-384. Standard intra- and extracellular solutions were used for patch-clamp experiments. Potency was measured by determining the increase in charge carried over 10 ms.
- **Animals.** *Scn1a*$^{+/-}$ mice were generated as described previously (Miller et al., Genes Brain Behav 2014).[1]
- **Brain Slice Preparation.** 400 µm parasagittal cortical brain slices were prepared from >P21 mice using standard procedures (adapted from Tai et al., PNAS 2014).[2] The slices maintained in a storage chamber with fresh artificial cerebrospinal fluid (aCSF) containing (in mM): 125 NaCl, 25 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 2 $CaCl_2$, 2 $MgCl_2$, 10 D-glucose, pH 7.3, osmolarity adjusted to ~306 mOsm using sucrose. All solutions were saturated with 95% O_2 and 5% CO_2.
- **Electrophysiological Recordings in Brain Slices.** Whole-cell current-clamp recordings were made in cortical layer 5. All recordings were done at room temperature (20-22°C). Fast-spiking interneurons were identified by their characteristic fast-spiking pattern, and confirmed *post hoc* by single-cell RT-PCR.

RESULTS

Potency and Selectivity of XPC-8770

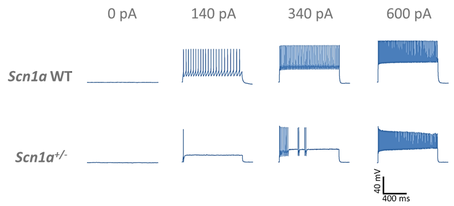


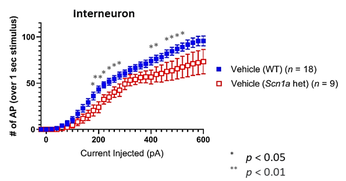


Compound	$Na_V1.1$ EC_{50} (µM)	$Na_V1.6$ EC_{50} (µM)	$Na_V1.2$ EC_{50} (µM)	$Na_V1.5$ EC_{50} (µM)	Selectivity $Na_V1.1/1.6$
Dominant Channel Expression	CNS: Inhibitory Interneurons	CNS: Excitatory Neurons	CNS: Excitatory Neurons	Heart: Cardiomyocytes	
XPC-8770	0.040	>30	>30	>30	>750

- XPC-8770 selectively potentiates hNa$_V$1.1 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.

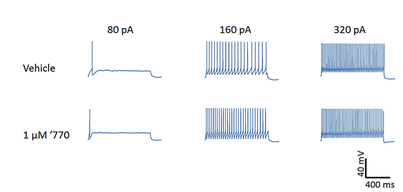
Shift in Rheobase and Decreased Maximal Firing Rate in *Scn1a*$^{+/-}$ vs. Wild Type (WT) Inhibitory Neurons

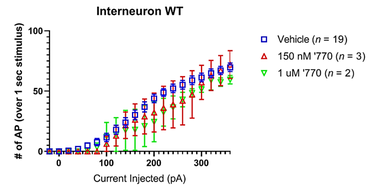




- When brain slices from wild-type mice and *Scn1a*$^{+/-}$ mice are compared, a shift in rheobase and decreased maximal firing rate in *Scn1a*$^{+/-}$ inhibitory neurons is observed.

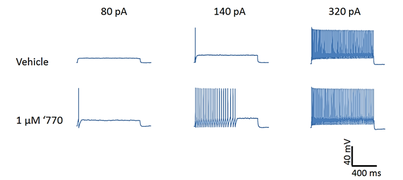
XPC-8770 Does Not Impact Firing of Wild-Type (WT) Inhibitory Neurons

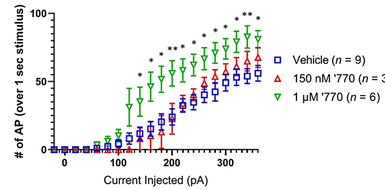




- In brain slices from wild-type mice, XPC-8770 does not impact the firing rate of inhibitory interneurons at concentrations of 150 nM and 1 µM.

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





- In brain slices from *Scn1a*$^{+/-}$ mice, XPC-8770 increased the firing rate of inhibitory interneurons at 1µM but not 150 nM.
- XPC-8770 treatment improved interneuron excitability, increasing maximum firing rate and preventing collapse of firing at high stimulus input.

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 *Scn1a*$^{+/-}$ interneurons to fire action potentials at high frequency.
- A small molecule pharmaceutical with this profile should enable reversal of the fundamental defect in Dravet Syndrome and may have utility in other neurologic indications where interneuron excitability is impaired.
- This profile provides a new, mechanistically differentiated, class of voltage-gated sodium channel potentiators with the potential to provide an improved therapeutic profile for Dravet Syndrome patients.

[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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