$Na_V1.1$ Selective Potentiators Normalize Inhibition/Excitation Imbalance and Prevent Seizures in a Mouse Model of Dravet Syndrome

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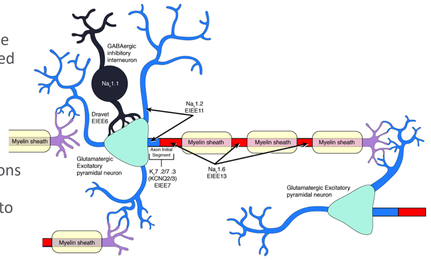
BACKGROUND

- 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, especially ion channels.
- We are pursuing brain penetrant small molecule potentiators of $Na_V1.1$ currents to allow oral dosing and titration 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.

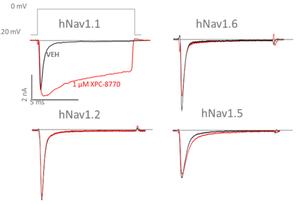
METHODS

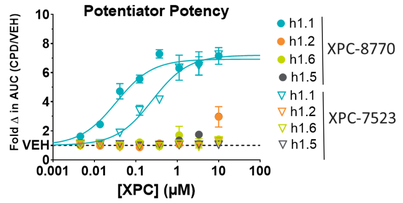
- **Voltage clamp electrophysiology** was used to assess the potency and selectivity of compounds in HEK cell lines stably expressing Na_V's on the Sophion Qube-384. Potency was measured by determining the increase in charge carried over 10 ms. Availability curves were generated by assessing current at test pulse following 500 ms prepulses to -120 to 0 mV in 10 mV steps. Error bars are ±SEM.
- **Animals**. *Scn1a*$^{+/-}$ mice and wildtype (WT) littermates 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. Fast-spiking interneurons were identified by their characteristic fast-spiking pattern. 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 ($X_{50\%}$).
- **Rotarod.** *Scn1a*$^{+/-}$ male mice were tested at a time point corresponding to the stabilization (P45). 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. Error bars are ±SEM.

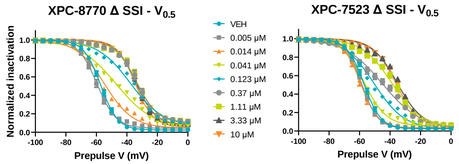
RESULTS

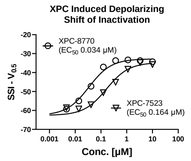
Potency, Selectivity and Mechanism of Action (MOA) of $Na_V1.1$ Potentiator CPD's

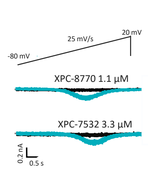
- XPC-8770 and XPC-7523 are representative compounds from two chemical subseries.
- The compounds selectively potentiate heterologously expressed h$Na_V1.1$ channels and spare potentiation and inhibition of neuronal channels $Na_V1.2$ and $Na_V1.6$ and cardiac channel $Na_V1.5$.
- The compounds slow open state fast inactivation and increase sodium influx upon depolarization.







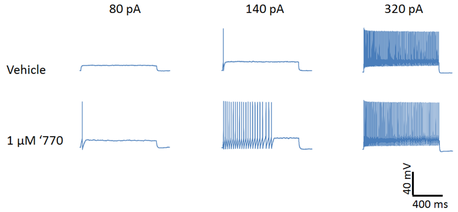


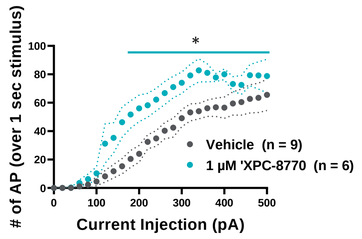


- These $Na_V1.1$ potentiators destabilize steady state inactivation and increase channel availability across a range of potentials close to neuronal resting membrane potentials.
- No effects on the voltage dependence of activation are observed.
- Compounds induced ramp currents that are related to increasing neuronal excitability.

$Na_V1.1$ Potentiator Compounds Selectively Increase Firing Rate of *Scn1a*$^{+/-}$ Inhibitory Neurons

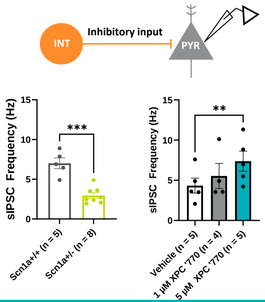
- In brain slices from *Scn1a*$^{+/-}$ mice, the area under the curve (AUC) for current injections >160 pA was significantly increased by XPC-8770 indicating a higher firing frequency of fast spiking inhibitory interneurons (P<0.05, Unpaired t-test).
- No significant effects were seen when XPC-8770 was applied to WT inhibitory interneurons.

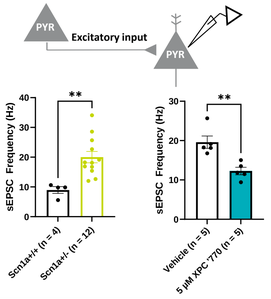




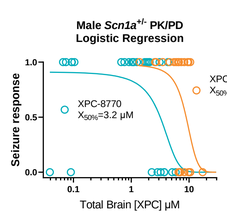
XPC-8770 Normalizes Spontaneous Post Synaptic Inhibitory and Excitatory Currents in *Scn1a*$^{+/-}$ Neurons

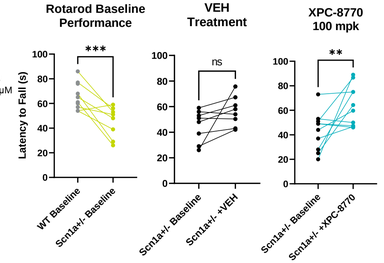
- *Scn1a*$^{+/-}$ mice display lower sIPSC frequency and higher sEPSC activity than WT (unpaired t-tests).
- XPC-8770 significantly increases sIPSC frequency (Two-way ANOVA, Tukey) and reduces sEPSC activity toward WT levels (unpaired t-test).





$Na_V1.1$ Potentiators Suppress 6 Hz Seizures and Improve Motor Performance in *Scn1a*$^{+/-}$ Mice





- *Scn1a*$^{+/-}$ 6 Hz seizure assay evokes seizures only in *Scn1a*$^{+/-}$ animals and confirms target engagement.
- Significantly improved performance on the Rotarod assay of *Scn1a*$^{+/-}$ mice suggests efficacy of XPC-8770 against non-seizure related symptoms such as motor dysfunction (Unpaired t-test).

CONCLUSIONS

- XPC-8770 and XPC-7523 are CNS penetrant, highly $Na_V1.1$ selective small molecule potentiators that impair fast inactivation, increase channel availability and increase Na^+ flux upon depolarizing inputs.
- This MOA increases impaired *Scn1a*$^{+/-}$ interneuron excitability and normalizes excitation/inhibition imbalance *Scn1a*$^{+/-}$ mice.
- The $Na_V1.1$ potentiators demonstrate target engagement in vivo by preventing seizures in a *Scn1a*$^{+/-}$ 6 Hz target engagement seizure model.
- The $Na_V1.1$ potentiator compound XPC-8770 improved Rotarod performance supporting the potential efficacy of this mechanism in non-seizure related symptoms.
- The $Na_V1.1$ potentiator profile 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.

REFERENCES

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.
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.

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