Molecularly Selective Na\textsubscript{v}1.1 Potentiators Increase PV+ Fast-Spiking Interneuron Excitability and
Restore Motor Performance in a Mouse Model of Dravet Syndrome

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RESULTS

**Potency, Selectivity and Mechanism of Action (MOA) of Na\textsubscript{v}1.1 Potentiator Compounds**

- **XPC-8770** and **XPC-7523** are representative compounds determined by chemical similarity.

- The compounds selectively potentiate heterologously expressed Na\textsubscript{v}1.1 channels and spare potencies to test inhibition of neuronal channels Na\textsubscript{v}1.2 and Na\textsubscript{v}1.6 and cardiac channel Na\textsubscript{v}3.5.

- The compounds slow open state fast inactivation and increase sodium influx upon depolarization.

**Methods**

- **Voltage clamp electrophysiology** was used to assess the potency and selectivity of compounds in HEK cell lines stably expressing Na\textsubscript{v}1.1 on the Soghroie-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. Cardiac channel profile was Eurofins Cardiac PROFILE.

- **Animals. Scn1a** mix and wildtype (WT) littermates were generated as described previously.

- **Brain Slice Preparation.** 400 µm parasagittal cortical brain slices were prepared from P21 mice using standard procedures.

- **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. iPSCs and iPSCs were recorded from layer 5 pyramidal cells in mice of ND/N, AXPS 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\textsuperscript{-/-} male mice by a 6 Hz stimulus for 3 seconds delivered through corneal electrodes and the CC19 was determined. Mice were stimulated at this current and placed in a plethysmograph chamber to monitor for the presence of a seizure characterized by paw clonus, tremor, piloerection, strabismus, and loss of balance. An animal was considered “protected” if none of these 4 behaviors occurred within 60 seconds. At least one of these behaviors was observed. Binaria seizure data were assessed with simple logistic regression to determine the concentration for 50% probability of protection (HC50). Error bars are SEM.

- **Rotated. Scn1a** male mice were tested at a point corresponding to the stimulation (P4). 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 20 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.

**Conclusions**

- **XPC-8770** and XPC-7523 are CNS penetrant, highly Na\textsubscript{v}1.1 selective small molecular potentiators that impair fast inactivation, increase channel availability and increase Na\textsubscript{+} flux upon depolarizing inputs.

- This MOA increases impaired Scn1a-** interneuron excitability and downstream excitation/inhibition imbalance in Scn1a-** mice.

- The Na\textsubscript{v}1.1 potentiators demonstrate target engagement in preventing seizures in a Scn1a\textsuperscript{-/-} 6 Hz target engagement seizure model.

- The Na\textsubscript{v}1.1 potentiator compound XPC-8770 improved Rotarod performance supporting the potential efficacy of this mechanism in non-seizure related symptoms.

- The Na\textsubscript{v}1.1 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.