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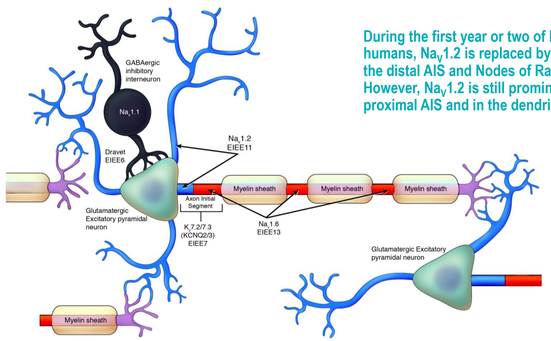
BIOMARIN SCIENTIFIC EXHIBIT
"Genetic Epilepsies – Updates in Science and Diagnosis"

Selective Sodium Channel Inhibitors and Potentiators; Pharmacology in Cortical Slices from Wild-Type and Dravet Mice

Informational Poster Prepared by Xenon Pharmaceuticals Inc.

BACKGROUND

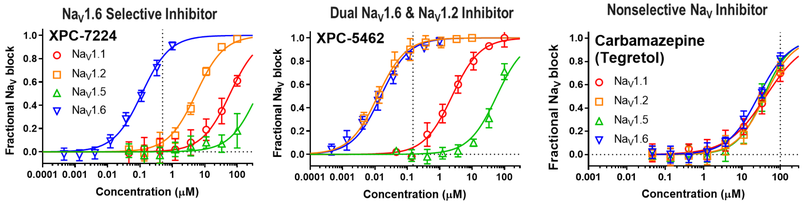
- An ideal anti-seizure medicine would inhibit excitatory circuits while stimulating inhibitory circuits.
- Voltage-gated sodium channel inhibitors (e.g. carbamazepine) are effective anti-seizure medications (ASMs) but these drugs inhibit the sodium channels that drive inhibitory interneuron firing ($Na_V1.1$) as well as those primarily linked to excitatory neuron firing ($Na_V1.2$ & $Na_V1.6$).
- Gain-of-function mutations in both *Scn8a* (encoding $Na_V1.6$) and *Scn2a* ($Na_V1.2$) cause early infantile epileptic encephalopathy in humans (EIEE13 & EIEE11, respectively).
  - Selective inhibitors of $Na_V1.2$ & $Na_V1.6$ that spare $Na_V1.1$ should provide improved ASMs.
- Loss-of-function mutations in *Scn1a* (encoding $Na_V1.1$) cause Dravet Syndrome (EIEE6) and nonselective sodium channel inhibitors can exacerbate seizures in Dravet Syndrome.
  - Selective Enhancers of $Na_V1.1$ should create specific therapy for Dravet Syndrome patients



During the first year or two of life in humans, $Na_V1.2$ is replaced by $Na_V1.6$ in the distal AIS and Nodes of Ranvier. However, $Na_V1.2$ is still prominent in the proximal AIS and in the dendrites.

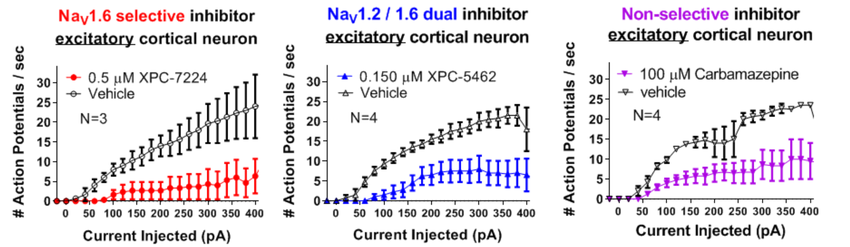
RESULTS

XPC-7224 Inhibits Only $Na_V1.6$; XPC-5462 inhibits Only $Na_V1.6$ & $Na_V1.2$



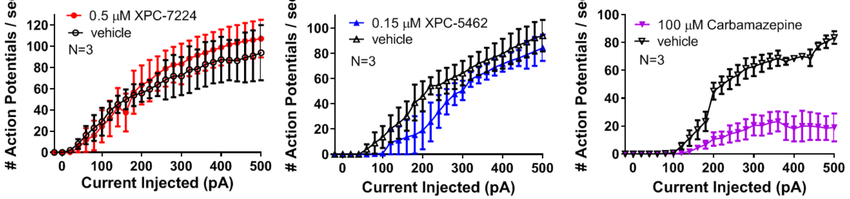
- XPC-7224 is highly selective for $Na_V1.6$.
- XPC-5462 blocks both $Na_V1.6$ and $Na_V1.2$; spares $Na_V1.1$ (Inhibitory Interneurons) and $Na_V1.5$ (Cardiac).
- Carbamazepine is similarly potent on all Na_V isoforms.
- For subsequent neuronal experiments we chose concentrations ~ 3X higher than the $Na_V1.6$ IC_{50} to target inhibition of ~ 80% of $Na_V1.6$ currents. The concentration used is indicated by the dotted vertical line on the selectivity graphs at the top:
  - XPC-7224, 0.5 μM
  - XPC-5462, 0.15 μM
  - Carbamazepine, 100 μM

Selective and nonselective inhibitors of Na_V's reduced action potential firing in cortical excitatory pyramidal neurons in mouse brain slices



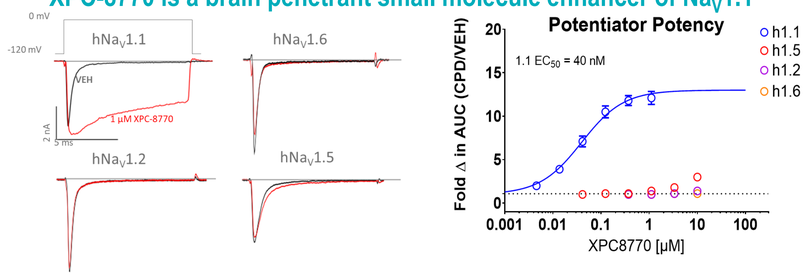
- All three test compounds, XPC-7224, XPC-5462, and Carbamazepine reduced action potential firing of excitatory glutamatergic pyramidal neurons to a significant and similar degree.

Only Inhibitors that Spare $Na_V1.1$ Spare Inhibitory Interneuron Firing



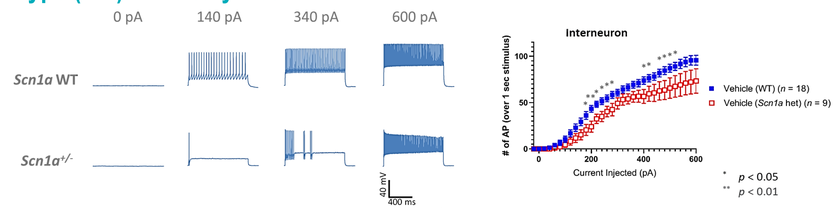
- Carbamazepine reduced action potential firing of inhibitory interneurons neurons to a significant and similar degree as in pyramidal neurons.
- $Na_V1.1$ sparing compounds, XPC-7224 and XPC-5462, had little effect on interneuron firing.

XPC-8770 is a brain penetrant small molecule enhancer of $Na_V1.1$



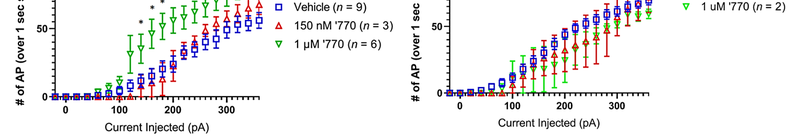
Compound	$Na_V1.1$ EC_{50} (μM)	$Na_V1.6$ EC_{50} (μM)	$Na_V1.2$ EC_{50} (μM)	$Na_V1.5$ EC_{50} (μM)	Selectivity $Na_V1.1/1.X$
Dominant Channel	Inhibitory Interneurons	Excitatory Neurons	Excitatory Neurons	Heart: Cardiomyocytes	
XPC-8770	0.040	>30	>30	>30	>750

Scn1a$^{+/-}$ Inhibitory Interneurons Fire Fewer Action Potentials Than 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 Enhances Firing of *Scn1a*$^{+/-}$ Inhibitory Interneurons But Does Not Change Firing of Wild-Type (WT) Interneurons



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

CONCLUSIONS

- Selective Inhibitors of specific sodium channel isoforms expressed in excitatory neurons, $Na_V1.2$ and $Na_V1.6$, enables selective reduction of action potential firing in those neurons, and prevents the simultaneous impairment of the activity of inhibitory interneurons.
- 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.
- Novel small molecule modulators of brain voltage-gated sodium channels have the potential to drive new personalized therapies for patients with both Gain and Loss of function mutations.
- Xenon is engaged in preclinical efforts to develop small molecule enhancers of $Na_V1.1$ for the treatment of Dravet Syndrome.

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