Poster
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Effects of BHV-7000 on Human iPSC-Derived Sensory Neurons From IEM Patients

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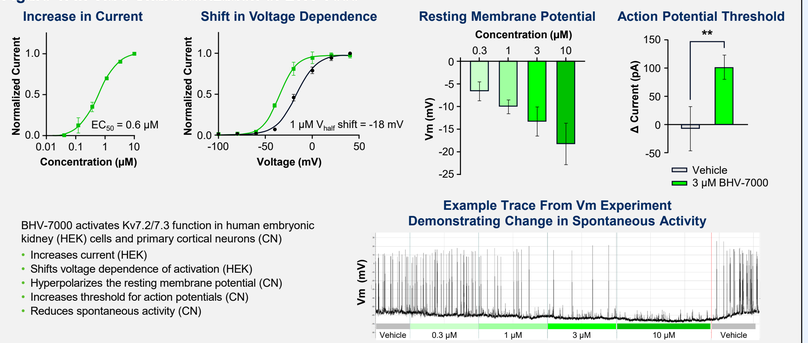
INTRODUCTION

- Chronic pain is highly prevalent and remains a significant unmet global medical need[1]
- Inherited erythromelalgia (IEM), caused by gain-of-function mutations in the NaV1.7 voltage-gated sodium channel, is a well characterized genetic model of chronic pain in which affected individuals experience burning pain in the extremities[2,3]
  - NaV1.7 is primarily expressed in the peripheral nervous system; the gain-of-function mutations in IEM produce hyperexcitability in peripheral sensory (dorsal root ganglion [DRG]) neurons (SNs)[2,3]
- To identify modulatory genes that confer pain resilience, we studied 2 IEM family cohorts, where 1 individual reported much less pain than other family members that share the same pathogenic gain-of-function NaV1.7 mutation[3,4]
  - Each pain-resilient individual carried a gain-of-function variant in Kv7.2 or Kv7.3, two potassium channels that stabilize membrane potential and reduce excitability[3,4]
- These gain-of-function Kv7.2 and Kv7.3 variants reduce DRG neuron excitability, suggesting that Kv7.2/7.3 activators may attenuate SN firing to alleviate pain,[3,4] thus prompting a search for new agents that target Kv7 as a potential new class of nonopioid pain therapeutics
- BHV-7000 is a potent and selective activator of the Kv7.2/7.3 voltage-gated potassium channel (**Figure 1**) and is in clinical development for epilepsy and neuropsychiatric disorders

Figure 1. In Vitro Characterization of BHV-7000



BHV-7000 activates Kv7.2/7.3 function in human embryonic kidney (HEK) cells and primary cortical neurons (CN)

- Increases current (HEK)
- Shifts voltage dependence of activation (HEK)
- Hyperpolarizes the resting membrane potential (CN)
- Increases threshold for action potentials (CN)
- Reduces spontaneous activity (CN)

**P ≤ 0.01.
Δ, change in; EC_{50}, half-maximal effective concentration; V_{half}, half-maximal activation voltage; Vm, membrane potential.

OBJECTIVES

- To determine if BHV-7000 can affect the firing rates of SN carrying a gain-of function NaV1.7 sodium channel mutation
- To determine if BHV-7000 can affect SN with NaV1.7 IEM mutations in a manner similar to Kv7.2/7.3 gain-of-function pain resilience mutations
- To determine if this "pain-in-a-dish" model can provide supportive evidence for a clinical trial in patients with IEM

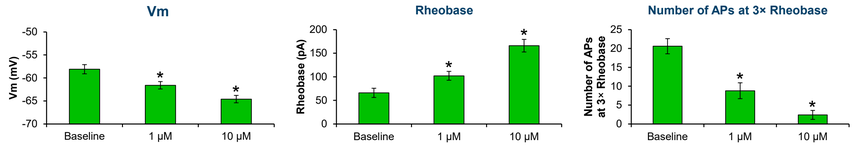
METHODS

- Standard patch clamp methods were employed to record from human-induced pluripotent stem cell (iPSC)–derived SNs (iPSC-SNs)[5]
- IEM iPSCs were generated from the blood samples of family members previously identified[3,4] to carry pathogenic mutations of NaV1.7 that cause IEM but with no Kv7 variants (IEM iPSC-SNs)
- Microelectrode array (MEA) recordings (Maestro, Axion Biosystems) were obtained to assess spontaneous firing as previously described[3,6]
- Current clamp recordings were obtained using an EPC 10 amplifier and the PATCHMASTER program (HEKA Elektronik)
- iPSC-SNs with a stable membrane potential were chosen for analysis
- Resting membrane potential (RMP) was determined immediately after switching into current clamp mode as the mean membrane voltage in the absence of current stimulation

RESULTS

- At 1 µM and 10 µM in control iPSC-SNs, BHV-7000 produced a hyperpolarization of -3.5 ± 0.4 and -6.5 ± 0.7 in RMP (mV) and 36.0 ± 3.4 and 100.0 ± 8.9 change in rheobase (pA) from baseline, respectively. At 3× rheobase, 1 µM and 10 µM reduced the number of action potentials (APs) by 52% and 87%, respectively, compared with control (**Figure 2**)
- Results recorded from IEM iPSC-SNs from a patient with IEM and carrying the Nav1.7-S241T mutation displayed robust spontaneous spiking activity as measured by MEA recordings (**Figure 3**)
- Analysis of the log_{10} of total spikes showed that BHV-7000 had an average half-maximal inhibitory concentration (IC_{50}) of about 75 nM, and the average maximal inhibition was about 92% (**Figure 4A, 4D**)
- In IEM iPSC-SNs from a patient with IEM carrying the NaV1.7-F1449V mutation, BHV-7000 had an IC_{50} value of about 70 nM and inhibited spontaneous activity by about 95% (**Figure 4B, 4D**)
- In current clamp recordings from NaV1.7-S241T iPSC-SNs, the average hyperpolarization of RMP in response to BHV-7000 1 µM was about -6 mV compared with vehicle control (**Figure 5**)

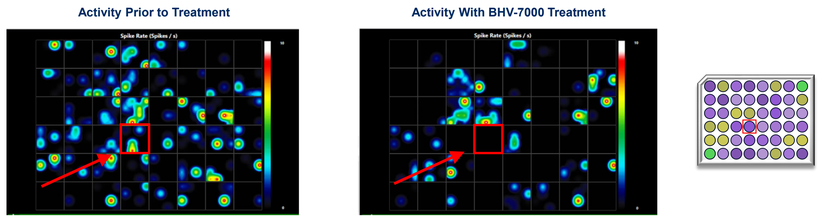
Figure 2. BHV-7000 Reduces RMP and Excitability in Control iPSC-SNs



	Δ Vm	Δ Rheobase	Δ Number of APs at 3× Rheobase
1 µM, mean ± SEM	-3.5 ± 0.4	36.0 ± 3.4	-11.8 ± 2.9
10 µM, mean ± SEM	-6.5 ± 0.7	100.0 ± 8.9	-18.2 ± 2.4

*Significant in paired *t* test compared with baseline. Error bars represent SEM.
Δ, change in; AP, action potential; iPSC-SN, induced pluripotent stem cell sensory neuron; RMP, resting membrane potential; SEM, standard error of the mean; Vm, membrane potential.

Figure 3. MEA Real-Time Visualization of Clone 300 of iPSC-SNs Treated With BHV-7000

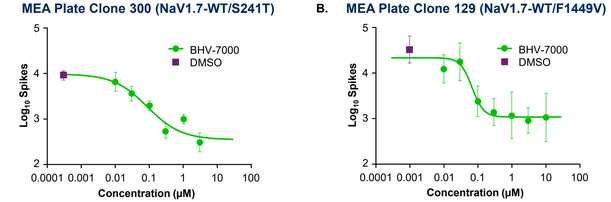


MEA real-time visualization of the activity of neurons plated on a 48-well plate

- Each well contains an array of 16 recording electrodes in a 4 × 4 grid
- The data are visualized as a color heatmap corresponding to the number of spikes detected for each electrode during the preceding second. Colors are updated every second
- Each well represents different concentrations of BHV-7000
- The mini plate layout on the right-hand panel is color coded to indicate the treatment groups; darker purple color represents higher compound concentrations applied
- The well indicated by a red arrow was exposed to BHV-7000 1 µM

iPSC-SN, induced pluripotent stem cell sensory neuron; MEA, microelectrode array.

Figure 4. Increasing BHV-7000 Concentration Leads to Decrease in Spike Activity



Spike counts were recorded over a 10-minute period and transformed into log_{10} (spikes). Groups are 5-6 wells (balanced). Vehicle is 0.1% DMSO.

C.

Clone 300	NaV1.7-WT/S241T, Kv7.2-WT/WT, Kv7.3-WT/WT
Clone 129	NaV1.7-WT/F1449V, Kv7.2-WT/WT, Kv7.3-WT/WT
Control Line	NaV1.7-WT/WT, Kv7.2-WT/WT, Kv7.3-WT/WT

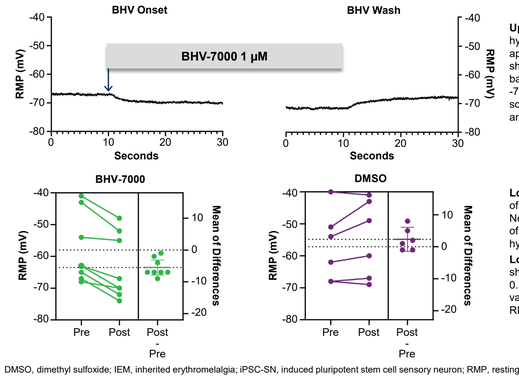
D.

	IC_{50} (µM)	Hill Coefficient	R^2 of Fit	Span	% Inhibition
Clone 300, MEA plate	0.046	2.3	0.75	1.3	95
	0.082	0.8	0.67	1.5	97
	0.098	1.5	0.47	0.8	85
Mean ± SEM	**0.075 ± 0.0154**	**1.5 ± 0.43**	**0.63 ± 0.083**	**1.2 ± 0.20**	**92 ± 3.7**
Clone 129, MEA plate	0.07	2.6	0.72	1.3	95
	0.10	2.7	0.83	1.8	98
	0.31	11.6	0.76	1.6	96
Mean ± SEM	**0.16 ± 0.075**	**5.6 ± 2.98**	**0.77 ± 0.032**	**1.6 ± 0.15**	**96 ± 0.8**

DMSO, dimethyl sulfoxide; IC_{50}, half-maximal inhibitory concentration; MEA, microelectrode array; SEM, standard error of the mean, WT, wild type.

Figure 5. BHV-7000 Hyperpolarizes Resting Membrane Potential From IEM iPSC-SN in Current Clamp

iPSC-SN Clone 300 NaV1.7-WT/S241T, Kv7.2-WT/WT, Kv7.3-WT/WT



Upper: Example trace showing the hyperpolarizing shift of RMP in response to application of BHV-7000 1 µM. This cell showed a stable RMP of -67 mV during the baseline period and a clear shift of RMP to -72 mV in response to BHV-7000. After some time, BHV-7000 was washed away and the RMP depolarized back.

Lower left: Combined before and after shifts of RMP in response to BHV-7000 1 µM. Nearly every cell showed a hyperpolarization of RMP and a significant average hyperpolarization of 5.5 mV (n = 8).

Lower right: Combined before and after shifts of RMP in response to vehicle control 0.1% DMSO. The shifts were small and variable and the 2.3 mV depolarizing shift of RMP was not significant (n = 6).

DMSO, dimethyl sulfoxide; IEM, inherited erythromelalgia; iPSC-SN, induced pluripotent stem cell sensory neuron; RMP, resting membrane potential; WT, wild type.

CONCLUSIONS

- This study demonstrated that BHV-7000 hyperpolarized the RMP, increased the rheobase, and decreased the AP firing rate at 3× rheobase from human iPSC-SNs using standard patch clamp recordings
- In IEM iPSC-SNs from 2 patients, MEA recordings demonstrated potent inhibition of spontaneous spiking with BHV-7000. The reduction in spiking activity is consistent with activation of Kv7.2/7.3 channels
- In addition, current clamp recording from 1 IEM iPSC cell line demonstrated that BHV-7000 hyperpolarized iPSC-SN RMP
- BHV-7000 reduced activity in a concentration-dependent manner with submicromolar efficacy
- These results implicate Kv7.2/7.3 channels as effective modulators of SN excitability, with and without sodium channel mutations, and suggest that BHV-7000 can specifically target Kv7.2/7.3 currents to reduce excitability in SNs, with the potential to be an effective treatment toward pain relief in patients with IEM

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DISCLOSURES: SD and **KP** are employed by and hold stock/stock options in Biohaven Pharmaceuticals. **ME**, **XC**, **SD-H**, and **SGW** have nothing to disclose.

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