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# Pharmacological Characterization of BHV-7000, a Novel and Selective Activator of Kv7.2/Kv7.3 Channels, Using All-Optical Electrophysiology


**Luis A. Williams, PhD[1]; Hongkang Zhang, PhD[1]; Joshua Meier, BS[1]; Jennifer Grooms, BS[1]; Steven J. Ryan, PhD[1]; Benjamin Harwood, PhD[1]; James J. Fink, PhD[1]; Caitlin L. Lewarch, PhD[1]; Owen B. McManus, PhD[1]; Graham T. Dempsey, PhD[1]; Steven Dworetzky, PhD[2]**

[1]Quiver Bioscience, Cambridge, MA; [2]Biohaven Pharmaceuticals, New Haven, CT


## INTRODUCTION

- Epilepsy is one of the most common chronic neurological conditions[1,2]
- BHV-7000 is a novel and selective activator of Kv7.2/Kv7.3, a key ion channel involved in neuronal signaling and regulating hyperexcitability in epilepsy[3,4]
  - In preclinical studies, BHV-7000 showed minimal gamma-aminobutyric acid type A ($GABA_A$) receptor activation and exhibited potent antiseizure efficacy in the maximal electroshock seizure (MES) model without negatively impacting neurobehavior or motor function[3]
  - The pharmacodynamic activity of BHV-7000 in the brain of healthy adults was demonstrated in a phase 1 study by dose-dependent increases in electroencephalograph spectral power[5]
- In phase 1 studies in healthy adults, BHV-7000 was well tolerated; the most common adverse events with multiple-ascending doses were headache and back pain[6]
- BHV-7000 is in clinical development for focal-onset seizures, generalized seizures, and mood disorders[7,8]
- In this study, we used an *in vitro* all-optical electrophysiology platform to further characterize the mechanistic activity of BHV-7000

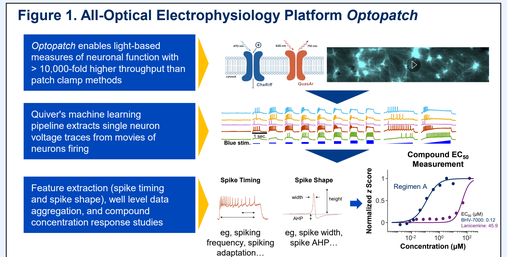
## OBJECTIVE

- To characterize the mechanistic activity of BHV-7000 under different treatment regimens and stimulus conditions

## METHODS

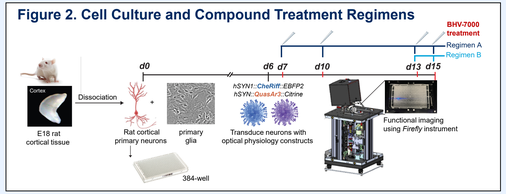
- The acute and chronic pharmacological effects of BHV-7000 on the neuronal excitability of primary rat cortical neurons were evaluated using the all-optical electrophysiology platform *Optopatch* (**Figure 1**)
  - *Optopatch* measures neuronal activity with single-cell and single action potential resolution and uses machine learning to profile drug interactions for drug discovery optimization[9,10]
  - *Optopatch* comprises **CheRiff**, a blue light-activated channelrhodopsin (voltage actuator), and **QuasAr**, an archaerhodopsin fluorescent voltage indicator. DNA constructs encoding these 2 proteins can be expressed in excitable cells such as neurons to enable simultaneous stimulation and detection of their electrical activity using light[9-11]
- Two different treatment regimens were evaluated in 2 independent experimental rounds using a 384-well assay format (**Figure 2**):
  - Chronic Regimen A comprised multiple compound treatment interventions during culture day (day *in vitro* [*DIV*] 7, 10, 13 (48 hours prior to measurements) and acute compound addition at *DIV*15
  - Regimen B used only 2 compound interventions: 48 hours and acute treatment prior to optical physiology measurements
- The stimulus protocol was composed of multiple "epochs" of differing stimulus shapes, intensities, and durations
- Half maximal effective concentration ($EC_{50}$) values were based on common features with unique characteristics providing additional mechanistic insight of compound interaction with the Kv7 channels

### Figure 1. All-Optical Electrophysiology Platform *Optopatch*




AHP, after hyperpolarization; $EC_{50}$, half maximal effective concentration; stim, stimulation.

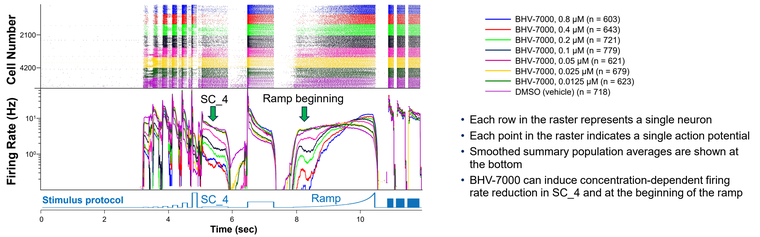
### Figure 2. Cell Culture and Compound Treatment Regimens




## RESULTS

- Functional measurements were made from > 200,000 individual neurons under 2 different treatment regimens
- Concentration-dependent dampening of neuronal excitability under gentle stimulus levels and increasing rheobase under ramp stimulus with an $EC_{50}$ of ≈ 100 nM were observed
  - These were consistent with the brain exposures-determined $EC_{50}$ in the BHV-7000 preclinical MES studies[3]
- BHV-7000 induced a concentration-dependent firing rate reduction in both long duration stimulus with low amplitude depolarization and the beginning of the ramp stimulus (**Figure 3**)
- Both treatment regimens showed similar $EC_{50}$ values, although more functional features tended to be altered under the longer chronic Regimen A (**Figure 4**)
- Additional analyses of the BHV-7000 functional data focused on select, shared *Optopatch* features that are impacted across several known antiseizure medicines (ASMs) to provide additional translatability to *in vivo* data (**Figures 5** and **6**)
  - The functional feature staircase 4 (SC_4)_*frequency_propZero* defined the fraction of neurons staying completely silenced during the long, gentle blue stimulus step SC_4

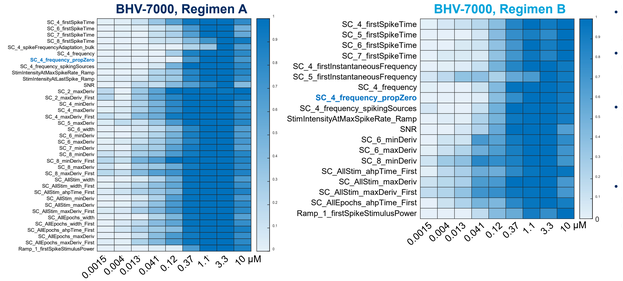
### Figure 3. Spike Raster Showing the Effects of BHV-7000 (Chronic Regimen A)




- Each row in the raster represents a single neuron
- Each point in the raster indicates a single action potential
- Smoothed summary population averages are shown at the bottom
- BHV-7000 can induce concentration-dependent firing rate reduction in SC_4 and at the beginning of the ramp

DMSO, dimethyl sulfoxide; SC_4, staircase 4.

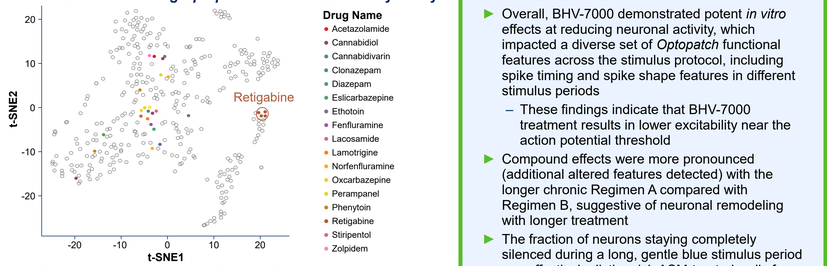
### Figure 4. Heatmaps of Functional Features Altered by BHV-7000




- Heatmaps show common features between BHV-7000 and riluzole treatment
- Feature selection was based on *z* score calculation: [mean(compound)-mean(DMSO)]/std(DMSO)
- Selection criteria: |z score| > 3 for a given feature in both BHV-7000 and riluzole in the same plate and was reproducible in all the replicate plates that passed QC
- Heatmap shows the normalized *z* score; each feature is normalized to its own maximum effect size

AHP, after hyperpolarization; DMSO, dimethyl sulfoxide; QC, quality control; SC, staircase; SNR, signal-to-noise ratio.

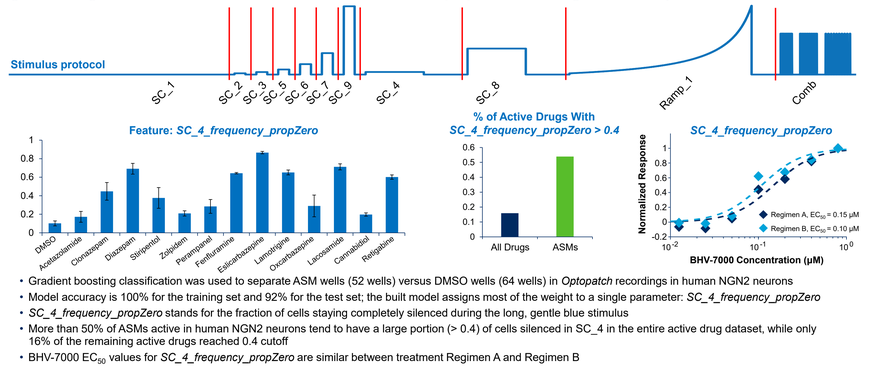
### Figure 5. t-SNE Plot of 400 CNS-Focused Drugs Measured in Human iPSC-Derived NGN2 Neurons Using *Optopatch* Intrinsic Excitability Assay




- Around 400 CNS-focused drugs were tested in 10-CRC using *Optopatch* assay in human iPSC-derived cortical excitatory (NGN2) neurons
- For each drug/dose, more than 500 *Optopatch*-determined functional features were reduced to 8 dimensions using autoencoder embeddings and t-SNE plot was used for visualization
- Only "active" doses with differential effects compared with DMSO are included
- ASMs are highlighted; most are represented by one dose

10-CRC, 10-point concentration response curve; ASM, antiseizure medicine; CNS, central nervous system; DMSO, dimethyl sulfoxide; iPSC, induced pluripotent stem cell; NGN2, neurogenin-2; t-SNE, t-distributed stochastic neighbor embedding.

### Figure 6. A Significant Common Functional Feature Identified in *Optopatch* Recordings From Multiple ASMs




- Gradient boosting classification was used to separate ASM wells (52 wells) versus DMSO wells (64 wells) in *Optopatch* recordings in human NGN2 neurons
- Model accuracy is 100% for the training set and 92% for the test set; the built model assigns most of the weight to a single parameter: *SC_4_frequency_propZero*
- *SC_4_frequency_propZero* stands for the fraction of cells staying completely silenced during the long, gentle blue stimulus
- More than 50% of ASMs active in human NGN2 neurons tend to have a large portion (> 0.4) of cells silenced in SC_4 in the entire active drug dataset, while only 16% of the remaining active drugs reached 0.4 cutoff
- BHV-7000 $EC_{50}$ values for *SC_4_frequency_propZero* are similar between treatment Regimen A and Regimen B

ASM, antiseizure medicine; comb, combined; DMSO, dimethyl sulfoxide; $EC_{50}$, half maximal effective concentration; NGN2, neurogenin-2; SC, staircase.

## CONCLUSIONS

- Overall, BHV-7000 demonstrated potent *in vitro* effects at reducing neuronal activity, which impacted a diverse set of *Optopatch* functional features across the stimulus protocol, including spike timing and spike shape features in different stimulus periods
  - These findings indicate that BHV-7000 treatment results in lower excitability near the action potential threshold
- Compound effects were more pronounced (additional altered features detected) with the longer chronic Regimen A compared with Regimen B, suggestive of neuronal remodeling with longer treatment
- The fraction of neurons staying completely silenced during a long, gentle blue stimulus period can effectively distinguish ASM-treated wells from dimethyl sulfoxide-treated wells in a parallel neuronal excitability assay using human-induced pluripotent stem cell-derived cortical excitatory neurogenin-2 neurons, suggesting this measure has utility for predicting ASM efficacy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**DISCLOSURES: LAW**, **HZ**, **JM**, **JG**, **SJR**, **BH**, **JJF**, **CLL**, **OBM**, and **GTD** are employed by Quiver Bioscience. **SD** is employed by and holds stock/stock options in Biohaven Pharmaceuticals.


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