Natural Product Compound Fractionization of the Lion's Mane Mushroom and HTS Analysis of its Effects on Multiple Models of Neuronal Disorders

Jocelyn Pena, Emery Smith, Richard Hawkins, Boyoung Lee, Louis Scampavia, Timothy Spicer

High-Throughput Molecular Screening Center, The Herbert Wertheim UF Scripps Institute for Biomedical Innovation & Technology

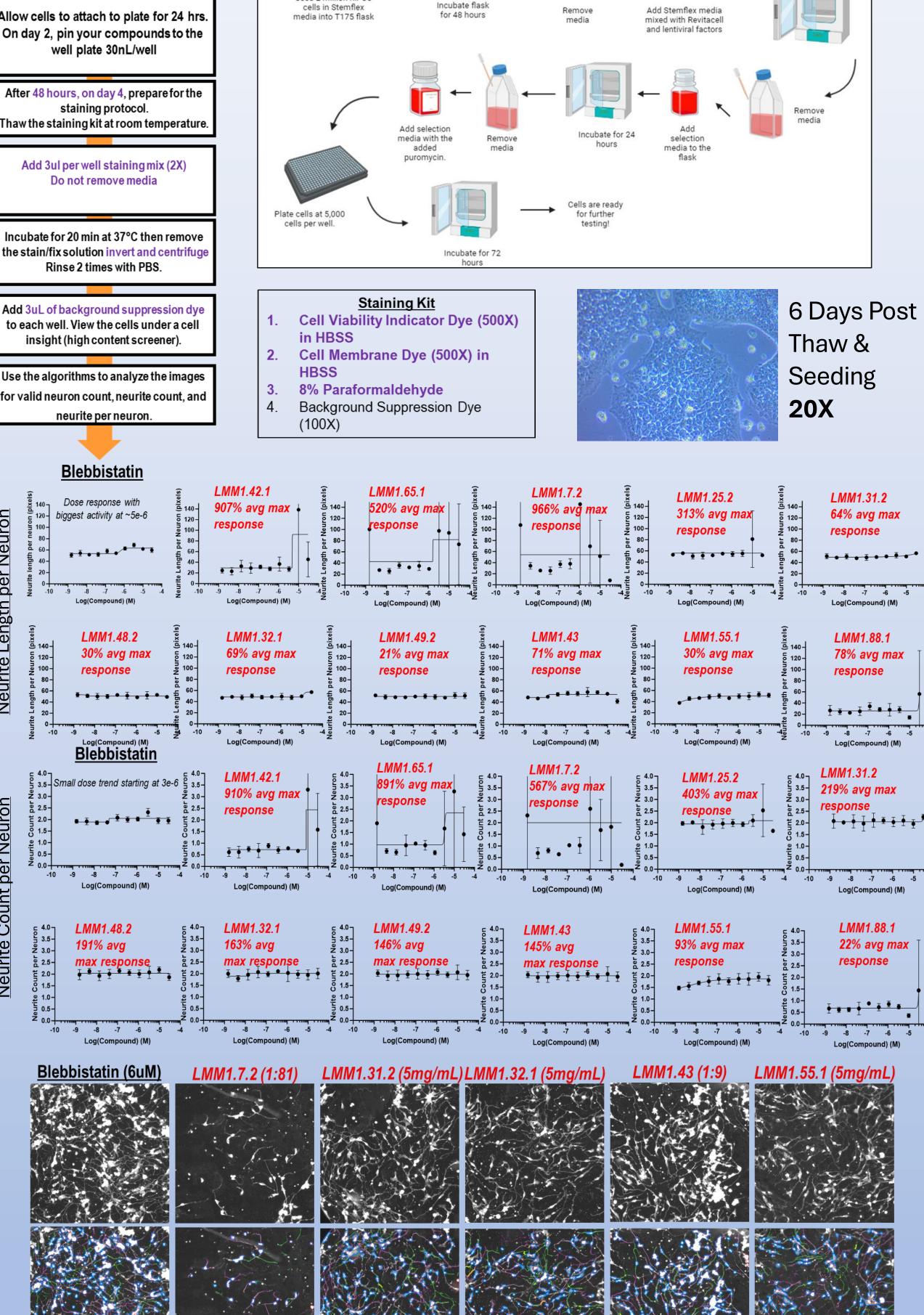
Overview

Neurodegenerative diseases have been taking the world by storm as modern medicine aids in increasing lifespans globally. Hericium Erinaceus, also known as Lion's Mane Mushroom, is consumed as both food and medicine worldwide. H. Erinaceus resides in the tooth fungus group and is native to Asia, Europe and North America. Studies have shown that there are many benefits to consumption of H. Erinaceus, ranging from relieving anxiety and depression all the way to the delay and protection against detrimental neurodegenerative diseases such as dementia and Alzheimer's. Lion's Mane mushroom is believed to contain polysaccharides, erinacines, hericenones, steroids, alkaloids, and lactones. This project was done to test the many intriguing capabilities of H. Erinaceus and determine which isolated components of the mushroom contribute to its specific capabilities. First, the team created 4 crude ethanol natural product extracts and tested them on primary neonatal pup neurons. The 4 fractions were used in assays to be analyzed via high content and luminescence analysis. The high content reader provided us with data on Valid Neuron Count per well, Total Neurites per well, as well as Total Neurite Count per Neuron. The luminescence analysis provided us with expression data analogous to increased BDNF or heterologous gene expression patterns associated with autism. From there CombiFlash Rf+ was done to accomplish the separation of mushroom components that were plated into 1536 well plates in a concentration response format. Altogether, 60 fractions of H. Erinaceus and analyzed using LC-MS. The 60 fractions were used in the same assay and monitored via high content analysis. The result of all procedures and outcomes is shown below.

BDNF Test of 4 Reflux Reflux Separation LMM Compounds Lion's Mane Mushroom Lion's Mane Mushroom Powder Separating Funnel Lower density liquid On day 1 Higher density liquid plate 500 or 1000 neurons > The positive control identified in the former LOPAC 24 Hour Protocol n a 1536 well plate in plating media ir <u>Test 1</u> testing shows the proper response a P-D-L coated plate. (3uL/well) > The 4 compounds RLU response is no higher than BDNF neurons were isolated Allow cells to attach to plate for 24 hrs and plated at 5,000 cells/well for 7 days On day 2, pin your compounds to the > The highest concentrations show some toxicity as well plate 30nL/well the signals decrease 100nL compound was added to the plate After 48 hours, on day 4, prepare for the By pintool and incubated for 24 hours Test 2 staining protocol Thaw the staining kit at room temperature media with the 30ul Nanoglo was added to the wells Add 3ul per well staining mix (2X) spun down and incubated for 10 minute Do not remove media - LMM2 - LMM3 - LMM4 - LMM4 DMSO High control High control The plate was read with the Viewlux the stain/fix solution invert and centrifuge -1 0 1 2 3 4 -1 0 1 2 3 4 Rinse 2 times with PBS. Log (ng/ml or nM) Log (ng/ml or nM) Add 3uL of background suppression dy CH2CL2 CH2CL2 Cell Viability Indicator Dye (500X) to each well. View the cells under a cell LMM1 insight (high content screener). extraction extraction extraction LMM2 Cell Membrane Dye (500X) in Internation. LMM3 LMM4 Use the algorithms to analyze the images DMSO 8% Paraformaldehyde or valid neuron count, neurite count, and Background Suppression Dye -1 0 1 2 3 4 5 -1 0 1 2 3 4 Log (ng/ml or nM) Log (ng/ml or nM) 48 Hour Protocol Thin Layer Chromatography BDNF neurons were isolated and plated at 5,000 cells/well for 7 days 1= ETOH extraction of Lion's F - - - - - Rf=0.94 - - Rf=0.92 Mane Mushroom (LMM1) - - Rf=0.83 _ _ Rf=0.76 100nL compound was added 48 hours C= Indol Control _____ - - Rf=0.66 **BDNF Total Neurites Per well** Rf= Retention factor - - - Rf = 0.50_____ -Rf=0.49 Thaw the staining kit at room temperature. • • • • • • • • • - Rf=0.40 **= - - - - - - - - Rf=0.31** Solvents: Remove the media from the well plates an HEXANE/ETHYL ACETATE contains the cell viability and membrane 10X High Content Images dye as well as 4% paraformaldehyde. (30uL ncubate for 20 min at 37°C then remove he stain/fix solution using the flick method Rinse 3 times with PBS. CombiFlash Rf+ Separation insight (high content screener). Jse the algorithms to analyze the images for valid neuron count, neurite count, and neurite per neuron **SYNGAP Test of 4 Reflux LMM** Tubes to 384 well plate Compounds **10X High Content** compounds were <u>Images</u> 48 Hour Protocol neat DMSO at Algorithm: ~5mg/mL for screening through **Neurites in pink and green** from Gavin's lab from DIV9 Nucleus in blue the LC-MS system. The initial EtOH Reflux fraction (#1) was experiments that were run. used to create these CombiFlash separations. the highest concentrations. After 48 hours, on day 4, prepare for the Run with solvents A/B: Hexane/Ethyl Acetate. Altered the %B of Ethyl Acetate: 1.5% after 10min a limited resource. 2. 20% around 28min Some of the 60 fractions show tendencies for neurite length increase per neuron, but the assay 3. 50% around 43min 4.100% around an hour 5. Switched to 100% MeOH after until the column was no longer showing brown coloration Want to know more about Log (ng/ml) Neurite Count Per Neuron

Induced Neurons 60

Fractions Test



Conclusions & Future Directions

- The 4 reflux compounds did not show any significant responses in the BDNF and Syngap
- The 5mg/mL 4 reflux compounds do show some toxicity as you can see the signal decrease at
- The compounds and cell density may not have been dialed in to show the best effects because the first 4 reflux compounds were not tested in a large dose format and the cells provided were
- needs to be repeated since the increase seen is marginal.
- BDNF & Syngap assays should be tested with the 60 fractions in dose format in the future.

the Herbert Wertheim UF Scripps Institute Molecular Screening Center, scan the first QR Code:





The Herbert Wertheim UF Scripps Institute for Biomedical Innovation & Technology