MUTATIONS CAUSING ALTERNATING HEMIPLEGIA OF CHILDHOOD DISRUPT NORMAL NEURAL NETWORK ACTIVITY PATTERNS

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Abstract:

Alternating Hemiplegia of Childhood (AHC) is a rare and debilitating neurological disorder affecting one in a million children. Greater than 80% of known cases are caused by *de novo* mutations in the *ATP1A3* gene. ATP1A3 is exclusively expressed in neurons and is thought to restore basal ionic concentrations in neurons after periods of fast firing. However, the underlying mechanism of pathogenesis of AHC-causing mutations is still unknown and there are currently no universally effective treatments. We are using the Axion Biosystems Maestro multi-well multielectrode array (MEA) platform to evaluate the spontaneous activity of neuronal cultures possessing AHC-causing mutations. MEAs are powerful tools for detecting spontaneous neuronal activity and are an emerging platform for identifying targeted therapies. We recorded neuronal activity 15 minutes each day to collect spike, burst, and network synchronicity data. Preliminary data from primary cortical cultures from one mouse model of AHC - Myshkin (I810N^{+/-}) - indicate aberrant organization of neuronal firing patterns in comparison to primary cultures from wild-type littermates. We hypothesize that aberrant activity is due to an imbalance of inhibitory and excitatory signals in the neuronal cultures. To test this hypothesis, we are evaluating the effect of several compounds with known mechanisms of action on the activity of cultured neuronal networks. Any identified compound that corrects the aberrant activity patterns will then be tested *in vivo* in the mouse models. Preliminary data indicate a strong shift in the response of mutant neuronal cultures to ATP, providing evidence for decreased ATPase activity in mutant cultures. These experiments illustrate the utility of the MEA platform in the evaluation of genetic mutations in rare neurological disorders and the response of such cultures to compounds. Furthermore, we are characterizing human embryonic stem cells (hESCs) possessing the monoallelic and biallelic D801N mutation and neurons derived from these stem cells in order to compare to data collected from the mouse model. This platform will be used to identify candidate targeted therapeutics for patients with AHC.

Experimental Design:

1. Wild-type C57BL/6J are mated with heterozygous Myshkin (I810N^{+/-}) mice to produce litters with 50% heterozygous offspring.
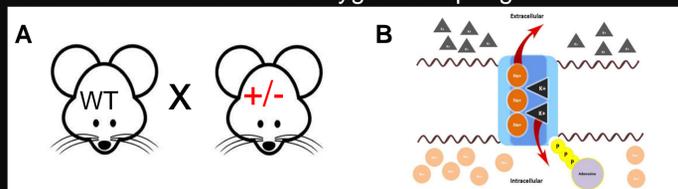
Figure 1: A) Mating scheme. B) Schematic of the function of the ATP1A3 protein in neurons

2. Post-natal day 0 pups are genotyped and brains are removed. Whole cortices are pooled together based on genotype, dissected, dissociated and plated at high density onto 48-well MEA plates.
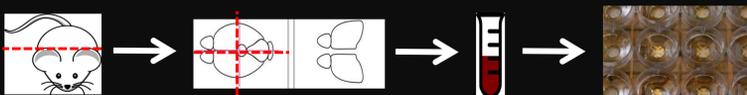
Figure 2. Schematic of mouse cortical dissections

3. Spontaneous activity of neural networks are recorded 15 minutes a day starting on day *in vitro* 3 (DIV3) until DIV19 on the Maestro MEA system.

Figure 3. Axion Biosystems Maestro Multielectrode Array, 48 well plate, and 4X4 electrode grid used to collect spontaneous activity data.

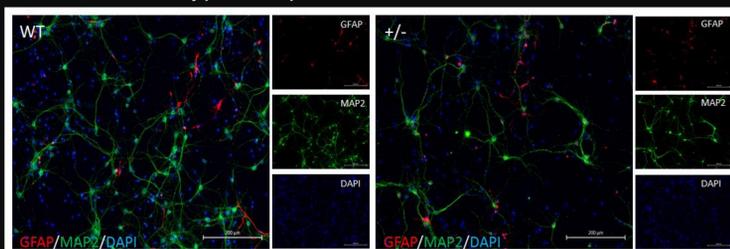4. Neural networks are stained and imaged to investigate potential differences in cell type composition.

Figure 4. Immunocytochemical images of neural networks. DIV15 networks on coverslips stained with GAD67, VGLUT1, and DAPI

5. Statistical tests are performed on a subset of the data during which activity is stable across a number of days (DIV11-DIV17; Figure 5)

6. Compounds, chosen based on known mechanism of action are added directly to each well (n=6) and spontaneous activity in response is immediately recorded (Figure7).
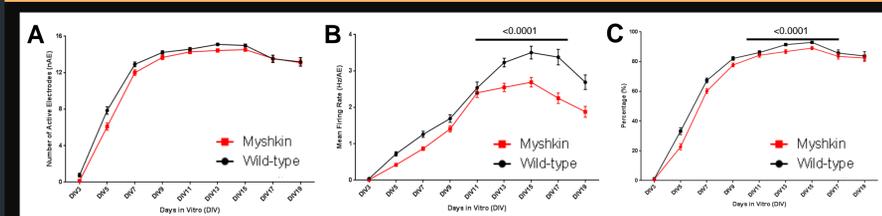
Results:

MEA:

Figure 5: Spontaneous activity feature comparisons. **A)** The number of active electrodes is equal between the wild-type and Myshkin groups. B) The mean firing rate and C) percent of spikes in bursts are significantly decreased in the Myshkin neural populations. (n=124; Mann Whitney U)
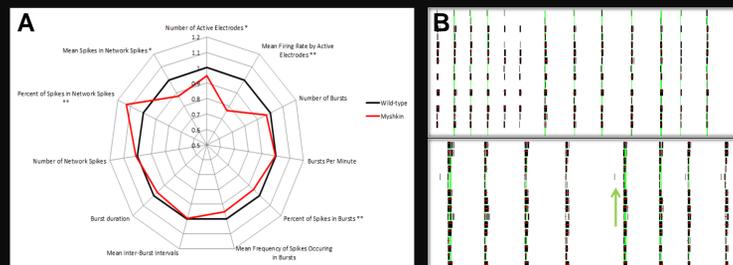
Figure 6: A) Spider plot illustrating the main spontaneous activity phenotype of the Myshkin mouse model in comparison to wild-type. B) Raster plots illustrating decreased synchronicity in mutant networks (wild-type above and mutant below)
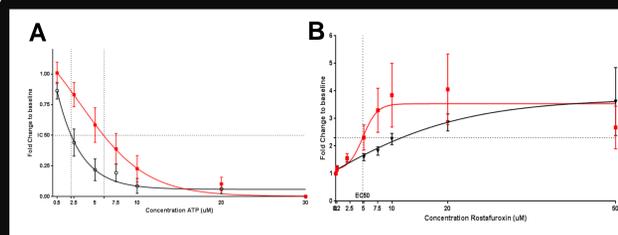
Figure 7: MFR response to drug treatment. A) Free ATP was shown to alleviate symptoms in one patient. Free ATP reduced the MFR of WT neurons at lower concentrations than for Myshkin neurons, illustrating a failure of mutant neurons to catalyze ATP. B) Rostafuroxin is a ouabain displacing compound that should increase ATPase activity. Rostafuroxin increases the MFR of mutant cultures at a lower concentration, indicating a possible rescue of the phenotype
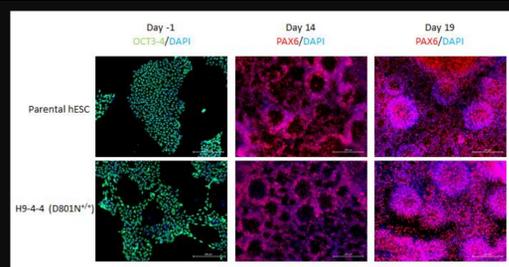
Stem Cell Characterization:

Figure 8: Stem cell derived neural networks using dual SMAD differentiation. There are no apparent difference in development of neuronal populations form stem cells.

Conclusions:

- Myshkin neural networks have lower mean firing rate than wild-type cultures and fail to fully synchronize in bursts but do have increased network spikes

- ATP addition results in a dose dependent decrease in firing of neural networks

- Wild-type neurons respond to a lower dose of ATP than Myshkin neurons.

- Rostafuroxin increases MFR at 10uM in mutant networks and may rescue the spontaneous activity phenotype observed on the MEA.

Future Directions:

- Isolate cellular subtype responsible for spontaneous activity phenotype using pharmacological manipulation and optogenetics

- Compare results of the Myshkin mouse model to that of the Mashlool mouse (D801N^{+/-}) model that possesses the most common AHC-causing mutation and the mutation present in the stem cell model

- Characterize stem cell derived neural populations and compare to primary neurons from mouse models
  - ➢ Morphology
  - ➢ Development
  - ➢ Network cell composition
  - ➢ Spontaneous activity

- Identify drugs capable of reverting any spontaneous activity phenotypes in primary and stem cell derived networks